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The effects of temperature on the physiology and locomotory behavior of the American lobster, Homarus americanus

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NOTE TO USERS

THE EFFECTS OF TEMPERATURE ON THE PHYSIOLOGY AND LOCOMOTORY BEHAVIOR OF THE AMERICAN LOBSTER, *HOMARUS AMERICANUS*.

BY

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DISSERTATION

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12-10-04
Date

DEDICATION

I would like to start off by saying that I actually intended to dedicate my dissertation to my parents. Then, during a recent conversation with my mom, she said "Well, just don't dedicate it to us... parents are SUPPOSED to support you!" So, I figured the least I could do was listen to her for once! But if not them, then who? Many people credit someone who got them interested in their field initially or who encouraged their pursuits. I have been interested in biology for as long as I can remember and, along the way, many people encouraged me. I had a great Biology teacher in high school who definitely started me on this path. I had an excellent undergraduate advisor, who was very inspirational and influential in helping me choose a graduate program. I have to include my parents because, among many other things, they were the first ones to take me to the ocean. So, how could I choose just one? I couldn't.

I started to think about WHY I had spent the last 8 years doing what I did...who was it for? I could only come up with one answer...it was for the lobsters! There they were, living their lives on the bottom of the ocean, entirely misunderstood! They weren't just little stenohaline, nocturnal beasts that move more when it's warmer and taste great with butter. They were complicated! Someone needed to speak up for them! I hope that's what I've accomplished and I'd like to dedicate this work to them...the little guys...the American lobsters of the Great Bay estuary system.

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I would like to thank the members of my committee, including Dr. Jenny Basil, Dr. Jan Factor, Dr. Larry Harris and Dr. John Sasner and especially my advisor, Dr. Winsor Watson, for all of their combined guidance, insight and support. I would also like to thank all the graduate and undergraduate members of the Watson lab that I have worked with over the years who it made an experience to remember. I need to especially mention Steve Jury, who was very helpful in teaching me the techniques used in my physiology experiments, and Susan Krull, who assisted me with aspects of both my physiological and behavioral experiments as part of her undergraduate work.

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ABSTRACT

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Suzanne M. Cooke

University of New Hampshire, December, 2004

Temperature has a pervasive influence on the physiology and behavior of all lobsters, and especially those found in habitats where temperature fluctuates the most. One population of lobsters undergoes a seasonal migration in the Great Bay estuary system in NH, entering the estuary in late spring and returning to the coast in the late summer and fall. This migration may be triggered by physiological or behavioral responses to seasonal temperature shifts.

The first set of experiments examined the effect of temperature on heart rate in order to further understand the influence of temperature on lobster metabolism. Heart rate was measured in intact lobsters, lobsters in which the cardioregulatory (CR) nerves had been lesioned and isolated lobster hearts. The heart rate of intact lobsters increased significantly and linearly with increasing temperature. Results from lobsters with lesioned nerves were not significantly different from the trend observed in intact lobsters. Isolated hearts did not show a significant increase in beat rate with increased temperature and ceased beating

between 20 and 25°C. These results indicate that, although some exogenous input must be involved in the heart rate response to temperature, the CR nerves are not the source of this input.

Temperature avoidance behaviors were examined for both summer- and winter-acclimated lobsters. Summer-acclimated lobsters avoided water that was warmer than 22.3°C +/-0.5 and there was a significant effect of sex, with male lobsters tolerating higher temperatures. Lobsters of all sizes exhibited similar responses. Winter-acclimated lobsters exhibited avoidance at 14.26°C +/- 1.1 with no influence of sex but a significant effect of size, with larger lobsters tolerating higher temperatures. Regardless of season, all lobsters exhibited avoidance after an approximately 8°C increase in ambient temperature. This suggests that an increase of this magnitude is aversive, possibly due to the simultaneous increase in rate of physiological processes that could place the lobster outside of its aerobic scope.

Activity levels were monitored in juvenile lobsters under both light and dark conditions over a range of ambient temperature conditions from 2.5-17.8°C. Lobsters traveled significantly farther in the dark than in the light and there was also a positive correlation between distance traveled and water temperature. There seem to be two distinct "activity states", with consistently low activity below 12°C and higher but more variable activity above 12°C. There was no significant effect of sex or size on activity.

These results correlate well with behaviors observed in the migratory population of lobsters in the Great Bay estuary system. Spring migrations

generally occur when temperatures are entering the range associated with the higher activity state. Movement out of the estuary could represent an avoidance response to high temperatures in the upper estuary in late summer. It appears as if lobsters may be avoiding warm water because the associated increase in metabolism may cause them to exceed their aerobic scope.

CHAPTER I

INTRODUCTION

The American lobster (*Homarus americanus*) is a large and common crustacean in the New England benthic marine community. Their distribution extends from Newfoundland and Labrador at the northern end to North Carolina in the south (Lawton and Lavalli, 1995). Both inshore and offshore populations have been identified, extending to the edge of the continental shelf. Cooper and Uzmann (1980) define inshore populations as those extending not more than 50km from coastal waters and offshore populations as those beyond 50km. Commercial fisheries target both populations, while inshore populations experience the additional pressure of recreational fishing.

The economic importance of this species has historically made it a focus of significant research efforts (Cobb and Phillips, 1980a&b; Factor, 1995). Estuarine lobster populations were largely overlooked in early studies due to the belief, expressed by Dall (1970, 1980), that lobsters were a fairly stenohaline species and would rarely enter estuaries. This view has since been refuted by numerous studies of lobster populations in habitats with variable salinity, including estuaries (Thomas, 1968; Thomas and White, 1969; Munro and Therriault, 1983; Reynolds and Casterlin, 1985; Vetrovs, 1990; Maynard, 1991; Jury *et al.*, 1994 a,b; Smith *et al.*,1998 a, b; Watson *et al.*, 1999; Howell *et al.*, 1999; Moriyasu *et al.*, 1999;

Little and Watson, 2003). The existence of a well-established, seasonal lobster fishery in the Great Bay estuarine system proves that lobsters have successfully exploited this habitat.

Examination of the distribution of lobsters in the Great Bay Estuary system in New Hampshire reveals a skewed sex ratio, with males outnumbering females in the upper reaches of the estuary by as much as 14:1 for the largest size class (>80mm CL) (Howell *et al.*, 1999). A similar distribution was described by Munro and Therriault (1983) for an estuarine lobster population in Canada and skewed sex ratios have been described for several other populations of lobsters, in some cases favoring males and in other cases favoring females (Skud and Perkins, 1969; Briggs and Mushacke, 1979; Karnovsky *et al.*, 1989). Interestingly, the ratio in the Great Bay Estuary seems to be affected by distance up the estuary as well as season and size class (Howell *et al.*, 1999). The sex ratio was the most skewed at upper estuarine sites and approached 1:1 at the coast. Seasonally, in the upper estuary, the largest ratio of males to females occurred in the spring and declined as the summer progressed, although always remained skewed in favor of males. Also, sex ratios were equal for the smallest size classes (up to 55mm CL) but became increasingly skewed for progressively larger size classes (up to >90mm CL). Howell *et al.* (1999) suggests that this distribution is a result of differential movement of larger males and females, possibly in response to temperature or salinity cues. To further understand the causal factors underlying this distribution pattern, additional research on the differential responses of male and female lobsters to environmental variables will be necessary.

The Great Bay Estuary

The Great Bay Estuary system includes Great Bay, Little Bay and the Piscataqua River. The estuary is heavily influenced by coastal tides and also receives input from seven large rivers and numerous small creeks. (Figure 1.1; Short, 1992). Due, in part, to this variety of inputs, the estuarine environment has high variability in multiple environmental factors, including salinity and temperature, on both a daily and a seasonal basis. Additionally, these factors can vary significantly as you move up into the estuary from the coast.

Salinity is highest and least variable at the coast and it decreases and becomes more variable as you move up the estuary. The highest salinities throughout the estuary are observed during the summer and fall, while minimums are reached during late winter and early spring in association with snow-melt runoff and spring storms (Short, 1992). Tide stage also affects salinity, with highest daily salinities observed during incoming tides. At all times, estuarine salinities are below that observed at the coast.

Temperature also shows high spatial, diurnal and seasonal variation within the estuary. Generally, maximum and minimum temperatures coincide with maximum and minimum salinities with the highest temperatures recorded during summer and fall and lowest temperatures occurring during the late winter and early spring (Figure 1.2; Short, 1992). The spatial variation in temperature is

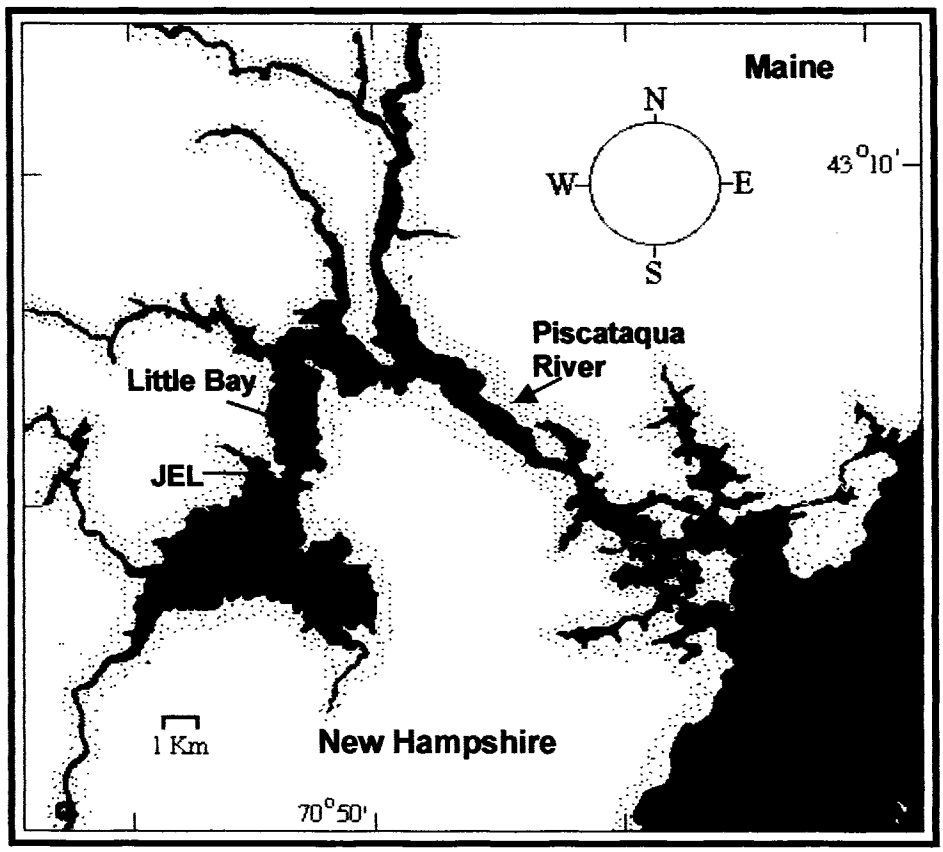


Figure 1.1. Map of the Great Bay Estuary System of New Hampshire. Arrow are used to indicate the locations of the UNH Jackson Estuarine Lab ("JEL") and the UNH Coastal Marine Lab ("CML"). Adapted from Short, 1992.

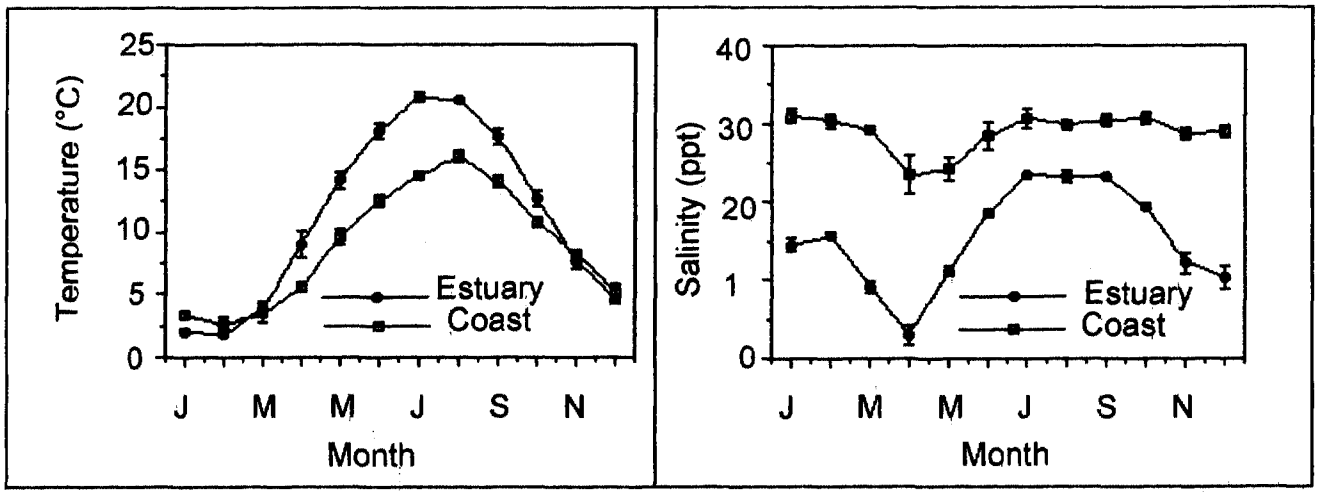


Figure 1.2. Month temperature and salinity averages from the UNH Jackson Estuarine lab ("Estuary") located on Adam's Point between Great Bay and Little Bay and the UNH Coastal Marine Lab ("Coast") located on Newcastle Island in the mouth of the Piscataqua River.

heavily influenced by season. The upper estuary gets both warmer in the summer and colder in the winter than coastal sites. Overall, the coastal end of the estuary shows less variation in temperature and salinity than the upper end of the estuary in Great Bay. Due to this spatial variation, tidal flushing has a strong diurnal influence on temperature regardless of season. During the summer, incoming tides bring cooler water into the estuary while in the winter they supply warmer water. These cycles occur twice daily in the estuary and result in temperature fluctuations of about 2-3°C. In the winter, tidal cycles could cause temperatures to range from ~3°C at low tide to ~5°C at high tide at the mouth of the estuary. In summer months, the range could be from 14°C at high tide to 17°C at low tide (Cooke, unpub; see Figure 3.2A for example). There are periods of time in the spring and fall when coastal and estuarine waters are nearly isothermic and tides have very little influence. Also, due to high levels of mixing and high flow rates, there is little vertical stratification in the estuary so conditions are similar regardless of depth (Short, 1992).

Challenges of an Estuarine Environment

The variability of the estuarine environment introduces challenges that must be overcome by species that attempt to exploit this area as a habitat. Dealing with changing salinity requires the ability to either osmoregulate or osmoconform across a broad range of salinities. Changing temperatures can greatly affect metabolic functions, both in terms of efficiency and in terms of demands that must be met. One study examining the abundance of zooplankton in North

Carolina estuaries found a significant correlation between temperature and abundance and concluded that temperature was the "single most important variable" in controlling zooplankton abundance in these estuaries (Mallin, 1991). Chen and Chia (1996) stated that, in an estuarine system, salinity and temperature are generally considered to be the major factors that limit the distribution of all invertebrates. Speaking specifically of homarid lobsters, Cooper and Uzmann (1980) stated that their geographic distribution is "broadly determined by their physiological tolerance to the major environmental variables" and then cited temperature and salinity as the main factors.

These environmental factors can be so limiting that for many species, only certain stages of life can tolerate the variability of estuaries, so estuarine use varies with developmental stage. In the mud crab, *Scylla serrata*, adults can exploit low salinity habitats but must return to full strength seawater to release their eggs. Then, after metamorphosis into juveniles, they are able to return to low salinity areas (Hill, 1974; Davenport and Wong, 1987; Hill *et al.*, 1992). This is likely to be related to changes in osmoregulatory ability during different life history stages. This type of shift has been demonstrated in the Florida stone crab (*Menippe mercenaria*). Larvae of this species exhibit an increased tolerance to low salinity conditions during zoeal stage 4, which has been suggested to coincide with the initiation of osmoregulatory ability (Brown *et al.*, 1992).

Charmantier *et al.* (2001) demonstrated that the ability of homarid lobsters to osmoregulate varies with developmental stage. Lethal salinity levels for larvae were between 15 and 17ppt while postlarvae and adults both exhibited lower

minimum tolerances (12ppt and 10ppt respectively). Additionally, they determined that tolerance to low salinities decreased at very low temperature (2°C). These values suggest that larval lobsters would have difficulty tolerating the conditions found within the estuary for most of the year and later developmental stages may have difficulties during the winter.

Estuarine Benefits

Since many species utilize the estuary as a habitat, there must be benefits that outweigh the challenges associated with estuarine life. Although the thermal regime of the estuary poses many challenges, it is also one of the greatest advantages that the estuarine habitat offers (Charmantier *et al.*, 2001). Higher water temperatures have been shown to be favorable for various aspects of life history such as molting, growth, mating, egg extrusion and development (Cooper and Uzmann, 1971; Cooper and Uzmann, 1980; Aiken and Waddy, 1986; Campbell, 1986; Lawton & Lavalli, 1995; Talbot & Helluy, 1995). Cooper and Uzmann (1980) observed differences in growth rates between inshore and offshore populations of American lobsters, which they attributed to differences in thermal environments. As much as half of the offshore population was observed to undergo seasonal migrations in order to maintain an ambient temperature greater than 8°C, whereas inshore lobsters experienced lower temperatures for at least half the year. Subsequently, offshore lobsters were observed to molt more frequently and express higher growth rates. It has also been demonstrated that warmer water leads to an earlier onset of reproductive maturity (Templeman,

1936a; Templeman, 1944; Aiken and Waddy, 1980; Aiken and Waddy, 1986; Fogarty, 1995; Little and Watson, 2003). Thus, lobsters that enter the estuary during the summer months may be more reproductively successful than lobsters that remain in coastal areas.

Another benefit the estuarine environment offers is the lack of larger, stenohaline marine fish, which reduces the threat of predation (Ennis, 1995; Charmantier *et al.*, 2001). Also, there is a reduction in both predation and competition due to the high availability of appropriate natural habitat and abundant crevices, which provide shelter (Thomas, 1968; Cooper *et al.*, 1975; Cooper and Uzmann, 1980; Wahle and Steneck, 1992; Lawton and Lavalli, 1995; Linnane *et al.*, 2000; Charmantier *et al.*, 2001).

Influence of Temperature on Metabolism

Historically, scientific attention has focused on the physiological effects of environmental variables on invertebrates in general and on economically important crustaceans in particular (see the following for reviews: Waterman, 1960; Bliss, 1983; Factor, 1995). Temperature has a strong positive correlation with metabolism in many species and can exert effects on other aspects of life history such as development, growth and behavior.

For many species of aquatic invertebrates, increased ambient temperatures are associated with increased oxygen consumption and increased ventilatory rates. This type of response has been seen in various species of shrimp (Ting, 1970; Liao & Huang, 1975; Liao & Murai, 1986; Chen and Nan,

1993), crabs (Dimock and Groves, 1975; Taylor, 1977; Taylor *et al.*, 1977; Gutermuth and Armstrong, 1989; Chen and Chia, 1996) and lobsters (McLeese, 1964; Mercaldo-Allen and Thurberg, 1987; Zainal *et al.*, 1992; Crear and Forteath, 2001). For example, Donnelly and Torres (1988) examined oxygen consumption in 14 species of midwater crustaceans from the Gulf of Mexico and calculated an average Q_{10} value of 2.20 over the range of 7-20°C. They concluded that temperature played a major role in controlling respiration within the entire community.

It has been suggested that some of this response may be the result of changes in hemolymph oxygen supply caused by temperature-induced structural changes in hemocyanin that affect the affinity for oxygen and by changes in dissolved oxygen content at different temperatures (Truchot, 1983; Taylor, 1981). However, structural changes to the hemocyanin molecule only occur after long-term exposure to altered temperatures (Mangum, 1983). Whiteley *et al.* (1995) demonstrated that European lobsters (*Homarus gammarus*) were able to compensate for changes in dissolved oxygen content brought on by sudden temperature changes by altering their ventilatory rate to maintain constant hemolymph oxygen concentration, so not all changes in ventilatory rate can be directly correlated with increases in oxygen consumption and thus metabolism. However, several studies showed increased ammonia production along with higher ventilatory rates at higher temperatures (Chen and Nan, 1993; Chen and Chia, 1996), which suggests an overall increase in metabolism as ambient temperatures increase.

Increases in heart rate in response to increased temperature have been demonstrated for many species of crustaceans including crabs (Ahsanullah and Newell, 1971; Florey and Kreibel, 1974; Spaargaren and Achituv, 1977; Burton *et al.*, 1980; Depledge, 1984; DeWachter and Wilkens, 1996; Aagaard, 1996; DeWachter and McMahon, 1996), crayfish (Bojsen *et al.*, 1998) and lobsters (Mercaldo-Allen and Thurberg, 1987; Nakamura *et al.*, 1994). However, unlike oxygen consumption and ventilatory responses, these increases are often non-linear. Frequently, heart rate shows a steeper rate of increase at both low and high temperatures, with a relatively temperature-independent range at intermediate temperatures where little change in heart rate is observed. Periods of steep increase in heart rate could represent periods of thermal metabolic compensation. Ectothermic animals are known to adjust their biochemical systems in order to compensate for temperature changes (Hochachka and Somero, 1984). In crustaceans, enzymatic activity has been observed to increase not only with increases in temperature but also with decreasing temperature, possibly associated with maintaining cellular oxygen supply and the efficiency of aerobic metabolic pathways at low temperatures (Lemos *et al.*, 2003). The increased enzyme activity would result in an increase in metabolic rate that could be reflected in increased heart rate. If this is true, it may be possible for crustaceans to exhibit behavioral responses to temperature that minimize these metabolic increases.

Temperature and Behavior

Most previous work directed at examining the effects of temperature on behavior of crustaceans fell into two general categories: avoidance/preference studies and activity studies. In avoidance studies, animals are subjected to an increasing or decreasing ambient thermal environment, generally within some sort of shelter but with a means to escape to more moderate conditions. The ambient temperature that initiates an escape response is considered to be the "avoided" temperature. In preference studies, which are somewhat more common, animals are provided with a natural or man-made gradient of temperatures through which they can move freely. The temperature where they choose to spend the majority of their time is considered to be their "preferred" temperature or thermal range. If, as suggested above, there is a range of temperatures where metabolism is most efficient, these types of studies can be used to examine whether or not a species is able to behaviorally thermoregulate to remain within that range.

Thermoregulatory behaviors in crustaceans have been repeatedly observed in both field and laboratory studies (Crawshaw, 1974; Galloway & Strawn, 1975; Loring and Hill, 1976; Hall *et al.*, 1978; Casterlin & Reynolds, 1979; Magnuson *et al.*, 1979; Reynolds and Casterlin, 1979; Mathur *et al.*, 1982; Crawshaw, 1983; Taylor, 1984; Lewis and Roer, 1988; Mundahl and Benton, 1990; Espina *et al.*, 1993; Kivivuori, 1994; Crossin *et al.*, 1998). Although the results from these studies vary, the common theme is the conclusion that animals are making locomotory choices that place them in a range of

temperatures where thermal metabolic compensation is minimized and metabolic rates can remain relatively low, while still allowing maximal growth. In this range, biochemical and physiological processes may occur at a maximal rate with a minimal expenditure of energy. Taylor (1990) found that growth rates in crayfish decreased when temperatures were either cooler or warmer than the preferred range. Within certain limits, acclimation to a new temperature regime will affect the physiological responses of poikilotherms (Prosser and Brown, 1961). Thus, it is likely that there is a wide range of temperatures where metabolism can function optimally after acclimation and, generally, enzymatic activity becomes adjusted to the current ambient conditions. If this were true, animals could make locomotory choices that keep them in similar ambient conditions as long as they remain within the larger acceptable range and avoid the elevated metabolism associated with thermal compensation.

There is some disagreement as to whether the animals are actively selecting a preferred temperature range or rather avoiding temperature extremes in their environment. This theory of orthokinesis was initially put forth by Fraenkel and Gunn (1961) and more recent research is inconclusive and suggests possible intraspecies differences. Early work with the crayfish *Orconectes immunis* (Crawshaw, 1974) fully supported the theory of orthokinesis. The crayfish showed no preference for a specific temperature and avoided the extremes of the gradient. It is also known that, contrary to what one might expect for an ectothermic organism, this species of crayfish becomes more active when cooled from 17°C to 3°C (Kerkut & Taylor, 1958), which could be a means to

behaviorally avoid extreme temperatures. An example of orthokinetic behavior in the field can be seen in the vertical movements of crab larvae in the water column, which occur in response to changing temperature (Forward, 1990). With increasing temperature, larvae descended and with decreasing temperature they ascended. This type of behavior would maintain the larvae in a stable thermal environment and avoid extreme changes in temperature.

Later studies stressed the importance of acclimation history on preference and avoidance, but still did not conclusively demonstrate preference rather than orthokinesis. Kivivouri (1994) showed that cold acclimated (5°C) crayfish selected and avoided different temperatures in a gradient than did those acclimated to 20°C. For both acclimation groups, a true selected temperature and a selected thermal range were calculated, which suggests a more complicated response by the animals than pure negative orthokinesis within a preferred range. In Espina (1993), acclimation temperature was shown to affect avoidance behavior but not the final preferred temperature, suggesting an even more limited role of orthokinesis and the existence of an inherent metabolic optima.

The blue crab, *Callinectes sapidus*, provides the only current example of a species that exhibits consistent thermal preference behaviors without exhibiting a specific preferred temperature range. Lewis and Roer (1988) examined the behavior of blue crabs that were free to move within a manmade lake, which acted as a cooling pool for a power plant. Regardless of the seasonal changes to the gradient found in the lake, the crabs consistently selected the warmest

areas of the lake unless it was over their lethal limit. However, the authors suggest that this may be an adaptation for dealing with the reduced salinity found in the lake because ion transport would occur more quickly at higher temperatures.

In regards to the American lobster (*Homarus americanus*), Reynolds and Casterlin (1979) demonstrated that the lobster exhibits consistent behavioral thermoregulation for at least a period of 6 days under laboratory conditions. During this time period, lobsters expressed both a true selected temperature (16°C) and a selected thermal range (15-21°C). After the initial 6 days of the experiment, however, this behavior seemed to degrade. A bimodal pattern of temperature preference appeared and the range of selected temperatures widened significantly (14-26°C). This was suggested to represent a shift from preference behavior to avoidance behavior, so that in the later days of the experiment lobsters were only avoiding the extreme temperatures in the gradient. Acclimation history has been demonstrated to affect patterns of behavioral thermoregulation in the lobster (Crossin *et al.*, 1998), with summer-acclimated lobsters selecting significantly different thermal environments than winter-acclimated lobsters. This seems to affect both avoidance and preference behaviors, and thus may be involved in directing seasonal migrations in this species.

Both small and large-scale seasonal movements have been linked to temperature in many crustacean species (Saila and Flowers, 1968; Cooper and Uzmann, 1971; Dennert & Van Maren, 1974; Rebach, 1974; Kanciruk and

Hernnkind, 1978; Munro and Therriault, 1983; Ennis, 1984; Karnofsky *et al.*, 1989; Lawton and Lavalli, 1995; Watson *et al.*, 1999). Often this leads to onshore-offshore movements to avoid seasonal extremes and optimize the thermal environment. This type of migration was identified in the American lobster by Cooper and Uzmann (1971). Based on tag-recapture data, they determined that a subset of offshore lobsters migrate onshore during the spring and early summer and then return to offshore sites in the late fall. They suggested that this migration was linked to temperature due to a strong correlation of lobsters found in association with bottom temperatures between 10 and 17°C. Watson *et al.* (1999) identified a similar pattern of movement in which coastal lobsters seasonally inhabit the Great Bay estuary system, entering in the late spring and returning to the coast in the fall. Neither of these studies addressed the mechanisms that might be controlling these migrations.

Some of the data on the relationship between temperature and migration for the American lobster is conflicting in nature and does not necessarily fully explain what is controlling migratory movements. Ennis (1984) observed activity levels to be positively correlated with temperature throughout the year, with increased activity observed beginning in late spring and early summer and decreased activity observed throughout the fall and early winter. The peaks in temperature and activity were offset somewhat, with temperature peaking in August and activity remaining high until mid-September. This pattern of activity would not be sufficient to explain a seasonal migration pattern. The increased activity in the spring could be correlated with an onshore migration but decreased

activity in the fall would not allow for the subsequent offshore trip. However, the population in this particular study was not noted to be migratory.

Karnofsky *et al.* (1989) observed a different seasonal activity pattern. In this study, activity levels did not correlate well with water temperature and were nearly reversed from what was observed by Ennis (1984). Activity was observed to decrease from May – August while water temperatures were increasing, and then increase again in the fall when temperatures began to drop. This pattern of activity provides more explanation for the initiation of the fall migration but decreased activity in the spring would not account for the onshore movements observed at that time.

Jury (1999) demonstrated that activity in general is temperature-dependent, at least in lobsters. Lobsters exposed to a range of ambient temperatures expressed a non-linear locomotory response. At very low temperatures and at very high temperatures, lobster locomotion was positively correlated with temperature. Over the range of intermediate temperatures, there was no correlation between increasing temperature and increasing activity. Rather, in this range of temperatures (~10-20°C) activity levels were stable and intermediate to what was observed in colder or warmer waters. This could suggest a pattern of increased activity to find an area with favorable thermal conditions. Then, the stable activity levels observed in the intermediate temperatures could act to maintain lobsters in a favorable location. Higher activity at higher temperatures could be a form of avoidance, which results in lobsters returning to more favorable conditions.

The need for further research

The economic significance of the American lobster fishery is tremendous. In 2002, the commercial and recreational lobster fisheries in the state of Maine alone landed 62,296,093 live pounds valued at approximately $207,307,460 (preliminary statistics from Maine Department of Marine Resources). In the United States and Canada, a total of 84,080 metric tons of lobster were landed in 2001 (Commercial Lobster Fisheries Profile, Department of Fisheries and Oceans, Canada, March 2004). Fluctuations in the population or shifts in sites optimal for use as habitat could greatly affect the efficiency, and thus the profitability, of the fishery. Also, random sampling to determine estimates of population abundance could be extremely inaccurate if the influence of abiotic variables is not taken into consideration. Although a large volume of research on both the physiology and behavior of this species exists, further research is necessary to fully elucidate the underlying relationships between abiotic factors, physiological parameters and expressed behaviors. Once we have a more complete understanding of these interactions, it may be possible to create models that would predict population fluctuations. Atrill *et al.* (1999) created a model that used multiple linear regression analysis of the relationship of estuarine crustacean abundance with many abiotic variables. Of the three species for which predictive models were created, temperature alone or temperature plus salinity were the only significant variables for *Carcinus maenas* and *Gammarus spp.* respectively. Thus, with a better understanding of the

physiological and behavioral responses to temperature, along with good long-term temperature records for the area, a similar model could be generated for *Homarus americanus* in the Great Bay Estuary System.

The studies presented in this thesis were intended to contribute to the body of knowledge of the effects of temperature on the physiology and behavior of the American lobster. By increasing this knowledge, it may be possible to explain complex population dynamics, such as the previously described skewed sex ratio observed in the Great Bay estuary system. This distribution has been suggested to originate in the sex-based differential response to environmental variables such as temperature or salinity (Howell *et al.*, 1999). In order to determine which environmental variables might play a role in establishing this distribution pattern, it is necessary to examine the physiological and behavioral effects of each variable independently of the others and subsequently consider interactions. My research focuses on how temperature is affecting the physiology and locomotory behavior of lobsters, and how this might contribute to the observed distribution.

In the first set of experiments, presented in chapter 2, I examined the effects of temperature on heart rate in the lobster and attempted to elucidate the controlling mechanisms for heart rate responses to temperature. There were several goals of this part of the study. First, I wanted to develop a thorough understanding of the response of the lobster heart to temperature over a wide range that encompassed most of what they might experience in their natural environment and, thus, most of their range of tolerance. This would allow a later

comparison of behavior over the same range of temperatures with underlying physiological parameters. Second, I wanted to determine whether temperature was influencing heart rate directly or indirectly. To accomplish this, I compared the effects of temperature on heart rate in intact animals with the effects of temperature on excised hearts. Finally, I wanted to elucidate the role of the cardioregulatory nerves in the heart rate response to temperature, so I repeated the study with animals in which these nerves had been severed.

Subsequently, I wished to address the relationship between this physiological variable and expressed behaviors. For the work presented in chapter 3, I focused on avoidance behaviors. Previous work by Crossin *et al.* (1998) had been unable to identify a temperature at which avoidance was exhibited for lobsters acclimated to winter temperatures. While winter lobsters may be eager to move into warmer waters, there must be an upper thermal limit at which temperatures would become aversive. Crossin *et al.* (1998) only exposed winter-acclimated lobsters to an 8°C increase in ambient temperature. I performed similar experiments in which the limit for ambient temperature was closer to the lethal maximum temperature for the species in an effort to elicit avoidance. Also, the work from this previous study focused solely on legal-sized lobsters (>82mm). I expanded this work to examine avoidance behavior in juvenile lobsters to see if size- or sex-based differences in acute tolerance could play a role in the distribution patterns of lobsters within the estuary.

The main goal of chapter 4 was to further examine the influence of temperature on lobster locomotor activity. Previous work on activity in American

lobsters (Jury, 1999) revealed a non-linear interaction of temperature and activity over the range of temperatures examined. Lobster activity was positively correlated with temperature both below 10°C and above 20°C but remained stable and decoupled from temperature from 10-20°C. Again however, this work was limited to adult, legal-sized lobsters. To expand upon these findings, I repeated the experiment using juveniles to determine if there were any ontogenic changes in behavior during this period, which could generate the unequal distributions based in the estuary.

CHAPTER II

EFFECTS OF TEMPERATURE ON THE HEART RATE OF THE AMERICAN LOBSTER, *HOMARUS AMERICANUS*

Introduction

The American lobster, *Homarus americanus*, is found in the western Atlantic Ocean from the Straits of Belle Isle, Newfoundland to Cape Hatteras, North Carolina. Across this range, lobsters are regularly exposed to ambient temperatures ranging from 0°C to 20°C (Aiken and Waddy, 1986). An even greater range in temperatures is experienced by populations that utilize estuarine habitats, such as the Great Bay estuary system in New Hampshire, where ambient temperatures can range from -2 to 27°C (Short, 1992). Not surprisingly then, it has been previously suggested that temperature is the most important abiotic influence on the behavior and physiology of lobsters and other crustaceans (Aiken and Waddy, 1986; Factor, 1995; Aagaard, 1996).

It is known that many crustaceans, including the lobsters found within the Great Bay estuary system, undergo some type of seasonal migration (Saila and Flowers, 1968; Cooper and Uzmann, 1971; Ennis, 1984; Pezzack and Duggan, 1986; Campbell, 1986; Gunderson *et al.*, 1990; Estrella and Morrissey, 1997; Watson *et al.*, 1999). Research suggests that seasonal migrations of crustaceans may serve to enhance growth rates and development. For example, Gunderson

et al. (1990) found that Dungeness crabs inhabiting an estuary exhibited a higher growth rate than crabs which settled in coastal areas. Similar results were obtained by Cooper and Uzmann (1971), who found that migratory populations of lobsters exhibited a higher growth rate than non-migratory populations, suggesting that the movements of the lobsters allowed them to maximize the time spent at temperatures more optimal to growth. Little and Watson (2003) found that female lobsters in an estuarine population, where ambient temperatures were higher, reached sexual maturity at a smaller size than female lobsters in an adjacent coastal population with lower ambient temperatures. Their results also suggested that there was significant mixing of these two populations, which might allow coastal lobsters to benefit from the warmer waters of the estuary and reach sexual maturity sooner.

An understanding of the physiological effects of temperature is necessary to evaluate the potential benefits of a seasonal migration. Early work by Templeman (1936b) showed that increased temperature greatly shortened the length of the larval period in lobsters. Aiken and Waddy (1986) found that temperature affects the timing of the onset of reproductive maturity as well, with lobsters that are exposed to higher temperatures reaching reproductive maturity at both a younger age and a smaller size. Temperature has been shown to exert a strong influence on oxygen consumption in all poikilotherms, including crustaceans. Increased temperature has generally been associated with increased oxygen consumption and ventilation rates (McLeese, 1964; Mercaldo-Allen and Thurberg, 1984; Gutermuth and Armstrong, 1989; Zainal *et al.*, 1992;

Crear and Forteath, 2001), which can be correlated with an overall increase in metabolism.

In decapods, heart rate generally increases with increasing temperature (Ahsanulah and Newell, 1971; Florey and Kreibel, 1974; Spaargaren and Achituv, 1977; deFur and Mangum, 1979; Burton *et al.*, 1980; Depledge, 1984; Mercaldo-Allen and Thurberg, 1987; Nakamura *et al.*, 1994; DeWachter and Wilkens, 1996; DeWachter and McMahon, 1996; Bojsen *et al.*, 1998). However, this relationship is often not linear. For example, Ahsanullah and Newell (1971) found that Q_{10} values for the heart rate of the shore crab (*Carcinus maenus*) were >2 at temperatures between 5 and 15°C, but decreased to as low as 1.55 at temperatures between 20 and 30°C. Both DeWachter and McMahon (1996) and DeWachter and Wilkens (1996) noted a similar but more extreme variation (acute Q_{10} values of ~3.0 at 4°C and 1.5 at 20°C) in studies involving the Dungeness crab (*Cancer magister*).

The lack of linearity in the crustacean cardiac response to temperature may suggest that there are certain temperature ranges over which physiological processes are optimized. In these ranges, metabolic rates would remain relatively low while still allowing maximal growth. Enzymes mediate energy production depending on the needs of an animal. In general, enzymatic activity increases with increasing temperature (Prosser and Brown, 1961). However, this is not always the case. In one species of shrimp (*Farfantepenaeus paulensis*), enzyme activity been observed to increase not only with increases in ambient temperature but also with decreasing temperature, possibly associated with

maintaining cellular oxygen supply and the efficiency of aerobic metabolic pathways at temperatures below the range of acclimation (Lemos *et al.*, 2003). Increases in enzymatic activity are indicative of thermal metabolic compensation and would result in an increase in metabolic rate. If this is true, it may be possible for crustaceans to exhibit behavioral responses to temperature that minimize these metabolic increases by maintaining the individual in an intermediate temperature range closer to their acclimation temperature.

Movements directed toward regions of ambient temperature within this range for a particular species could play a role in initiating or limiting seasonal migrations. For instance, if a particular species was within their aerobic scope and could minimize metabolic increases over the range of 10-20°C, this could result in a spring migration into an estuary since estuarine temperatures exceed coastal temperatures by late spring and throughout summer. In the fall and early winter, as estuarine temperatures dropped below coastal values, a migration out of the estuary could occur.

To date, little work has been done to elucidate the underlying control mechanisms for the crustacean cardiac response to temperature. Generally, the rate of the neurogenic crustacean heart is controlled by a combination of neural and hormonal inputs. The cardiac ganglion, which lies on the dorsal wall of the heart, receives input from the cardioregulatory nerves (Alexandrowicz, 1932; Maynard, 1953; Florey, 1960; Field and Larimer, 1975; Wilkens and Walker, 1992; Guirguis and Wilkens, 1995) and is the site of action for many extracts of the pericardial organs (Alexandrowicz, 1953; Alexandrowicz and Carlisle, 1953;

Maynard and Welsh, 1959; Belamarich and Terwilliger, 1966; Cooke, 1966; Berlind and Cooke, 1970; Cooke and Hartline, 1973; Grega and Sherman, 1975; Evans *et al.*, 1976; Florey and Rathmayer, 1978; Battelle and Kravitz, 1978; Beltz and Kravitz, 1983; Kingan and Titmus, 1983; Benson, 1984; Schwartz *et al.*, 1984; Siwicki *et al.*, 1987; Cooke, 1988; Dircksen and Keller, 1988; Wilkens and McMahon, 1992; Wilkens and Mercier, 1993; Kuramoto and Tani, 1994). It is currently unknown which of these sources, if either, is involved in the cardiac response to temperature.

The goals of this study were to describe how the heart rate of lobsters is affected by changes in ambient temperature over a range that they would normally experience and to examine a possible source of temperature-induced heart rate modulation. I examined the effects of temperature on the heart rates of intact lobsters, isolated hearts, and lobsters in which the cardioregulatory nerves have been severed. Results from intact lobsters provide basic physiological data on how lobsters are affected by temperature and also serve as a baseline with which to compare the results of the other two treatments. In an isolated heart preparation, all external inputs are removed, eliminating both neural and hormonal inputs and providing information on how the heart tissue itself is affected by temperature. Finally, data obtained from lobsters with severed cardioregulatory nerves allow a distinction to be made between the nervous and hormonal controls in an intact animal. Any component of the response that still occurs after severing these nerves is likely to be hormonal in origin. Conversely, any component of the response that is lost would be neural in origin.

Methods

Animals

Adult lobsters of both sexes, between 74 and 90 mm carapace length, were obtained using standard lobster traps located near either the Jackson Estuarine Laboratory in Durham, New Hampshire or the University of New Hampshire (UNH) Coastal Marine Laboratory in Newcastle, NH. All lobsters were held in a 90L-seawater aquarium (15°C, 32 ppt) in Rudman Hall on the UNH Durham campus for a minimum of 2 weeks and a maximum of 4 weeks prior to being used in an experiment.

Recordings from intact lobsters

A total of 25 adult lobsters (74-90 mm CL) were used for intact animal recordings. On the day prior to each experiment, a pair of wire electrodes (30 gauge Kynar wrapping wire) was inserted through the dorsal carapace along the midline of the thorax just anterior and just posterior to the heart. Electrodes were secured in place using a section of tape coated with fast-drying cyanoacrylate adhesive. The electrodes were connected to an impedance converter (model 2991, UFI, Morro Bay, CA, USA), and the resulting analog signal was digitized and displayed with a Maclab data acquisition system (ADInstruments, Colorado Springs, CO, USA) using the Chart (v. 4.01, ADInstruments) software.

In all experiments, lobsters were placed in a 38L aquarium containing a Plexiglas recording chamber (Figure 2.1). Aquarium temperature was controlled via an aluminum heat transfer coil connected to a refrigerated recirculating bath

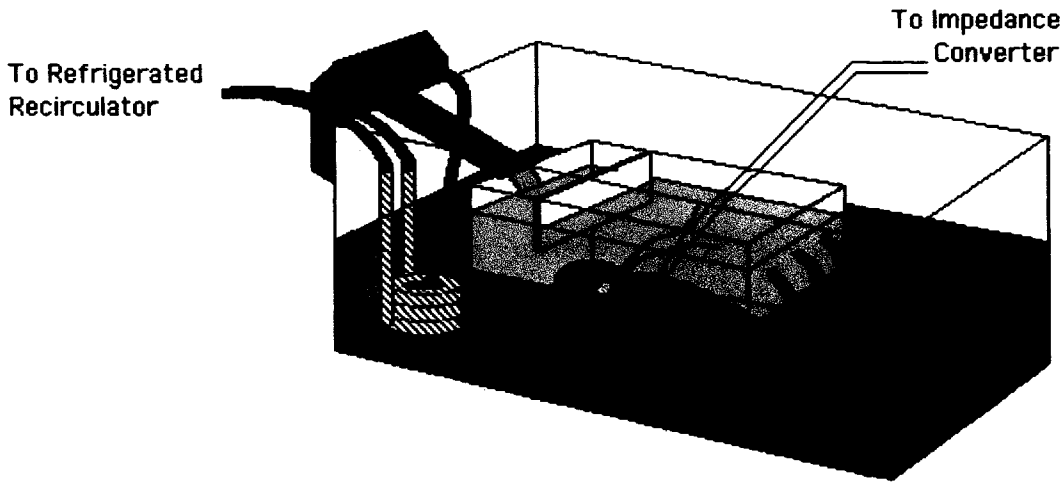


Figure 2.1. Aquarium and Plexiglas recording chamber used for trials involving intact animals. The water level within the recording chamber was maintained at a higher level than that of the surrounding tank in order to achieve a directional water flow from the heat transfer coil, through the chamber and back into the surrounding aquaria.

(model 900, VWR Scientific, Niles, IL USA). The aquarium was wrapped with fiberglass insulation and covered with a Styrofoam lid to minimize visual and auditory disturbances and to help maintain temperature control. At the beginning of the experiment the temperature of the tank was set to 5°C. The lobster was allowed to become accustomed to the tank overnight before the experiment began.

The heart rate was recorded for 20 minutes prior to increasing the temperature of the tank, by 5°C, at a rate of <0.1°C/min. It took about an hour for this thermal transition to take place. A second 20-minute section of heart rate data was recorded at 10°C and then the temperature of the tank was increased to 15°C. This process was repeated for every 5°C increment up to a maximum of 25°C. Three control trials were performed using the same protocol, but temperature was maintained at 15°C.

Isolated heart preparation

A total of 10 adult lobsters (>78mmCL) were used for isolated heart preparations. The dorsal 1/3 of the thorax was removed and pinned ventral side up in a shallow dish filled with cold lobster Ringer's solution (472mM NaCl, 10mM KCl, 16mM $CaCl_2$, 7mM $MgCl_2$, 10mM TRIS-HCl, 10mM Maleic acid, 11mM glucose (Govind and Lang, 1981)). Extraneous tissue was removed without damaging the pericardial membrane. The sternal artery was cannulated with a 1mm-diam. glass capillary tube. All other vessels were tied off with suture thread to maximize pressure output from the sternal artery. The glass capillary tube

extending from the sternal artery was connected to a pressure transducer (FT.03, Grass Instruments, Quincy, MA, USA). The pressure signal was amplified (model ETH--200, CB Sciences, Dover, NH, USA) and then digitized and displayed using the Maclab data acquisition system.

The dish containing the heart was placed in the same temperature-controlled aquarium used for the intact lobster experiments. The only addition to the setup was a small stand used to support the heart chamber so that its walls were slightly above the water level of the holding chamber. A glass bottle containing the Ringer's solution was partially submerged in the aquarium in order to maintain it at the same temperature as the water in the aquarium. This aerated Ringer's solution was perfused into the heart chamber throughout the experiment using a peristaltic pump (Variable flow mini pump, VWR Scientific). The seawater surrounding the heart chamber controlled the temperature of the fluid surrounding the heart, but did not mix directly with the Ringer's solution. A digital thermometer (VWR Scientific) was used to monitor the temperature of the water surrounding the heart. The protocol for temperature changes and data collection was the same as for intact lobsters. Three control trials were performed in which isolated hearts were maintained at a stable temperature (15°C) for a minimum of 6 hours and heart rate was continuously recorded.

Cardioregulatory nerve lesions

A rectangular section (2.5 x 1.0 cm) of the dorsal carapace directly above the heart was removed, taking care not to tear the pericardial membrane. A pair

of iris spring scissors was used to cut the membrane just posterior to the heart, through which the cardioregulatory nerves pass (Guirgus and Wilkens, 1995). The section of carapace was reattached and held in place with parafilm and a layer of tape secured with fast-drying cyanoacrylate adhesive. After completion of the lesion procedure, lobsters were allowed a minimum of 2 days to recover in a 15°C holding tank.

Cardioregulatory nerves are known to control cardiac responses to novel stimuli (Guirgus and Wilkens, 1995; Jury and Watson, 2000). Prior to undergoing the lesion procedure, all animals used in these experiments were subjected to several stimuli (light, touch, low salinity pulses) known to elicit a cardiac response. Only animals with a normal cardiac response to salinity (rapid decrease followed by an increase in rate) prior to lesioning were used in the experiment. After recovering from the lesioning procedure, the cardiac test was repeated. The lack of a bradycardia response during this second test was used as an indicator of a successful lesion. A total of 5 lobsters were successfully lesioned. These five animals were allowed another two days to recover prior to use in the temperature experiments. Subsequently, the temperature experiment described previously for intact animals was repeated with these 5 lesioned lobsters.

Data Analysis

In all preparations (intact lobsters, lesioned lobsters, isolated hearts), a 10-minute section of data was analyzed at each experimental temperature (5°C,

10°C, 15°C, 20°C and 25°C). Data for analysis were only taken from sections during which the temperature was stable. These data were used to determine average heart rates at each temperature for each treatment. All averages are presented as mean ± SEM. Statistical comparisons were done using the Mann-Whitney or Kruskal-Wallis tests for non-parametric data (Instat 2.01). Simple linear regression analysis was used to determine if the response of heart rate to temperature was linear, and to compare the response of intact lobsters to the response of lobsters with lesioned cardioregulatory nerves.

For control trials using intact lobsters and isolated hearts, an average heart rate was calculated for 10-minute subsamples of the data for each hour over a minimum of 8 hours.

Results

Intact lobsters

In intact lobsters, increases in water temperature led to an acceleration of the heart rate (Figure 2.2A). The average basal heart rate of intact lobsters at 5°C was 26.1 ± 2.1 BPM (n = 25). As temperature was increased from 5°C to 25°C, there was a significant increase ($p < 0.001$) in heart rate (Figure 2.3) and there was a strong correlation between heart rate and temperature ($r^2 = 0.72$). Q_{10} values were calculated for each 5°C interval (Table 2.1). These values indicate that, although heart rate increased with temperature over the range examined, increases in were greatest at the low end of the temperature range. In contrast, control lobsters (n = 3) maintained at 15°C showed no significant change in heart rate over the recording period of 6 hours ($p = 0.64$) and there was a significant difference between the heart rate of intact and control lobsters at each temperature >5°C.

Isolated hearts

Only isolated hearts with rates > 20 BPM at 5°C were included in the final analysis because lower rates were never seen in intact animals and could be indicative of damage to the heart from the isolation procedure. Using this criterion, 3 out of 10 isolated heart experiments were excluded from the final analysis.

Representative recordings from isolated hearts are presented in Figure 2.2C. The average basal rate for isolated hearts at 5°C was 26.8 ± 1.5 BPM

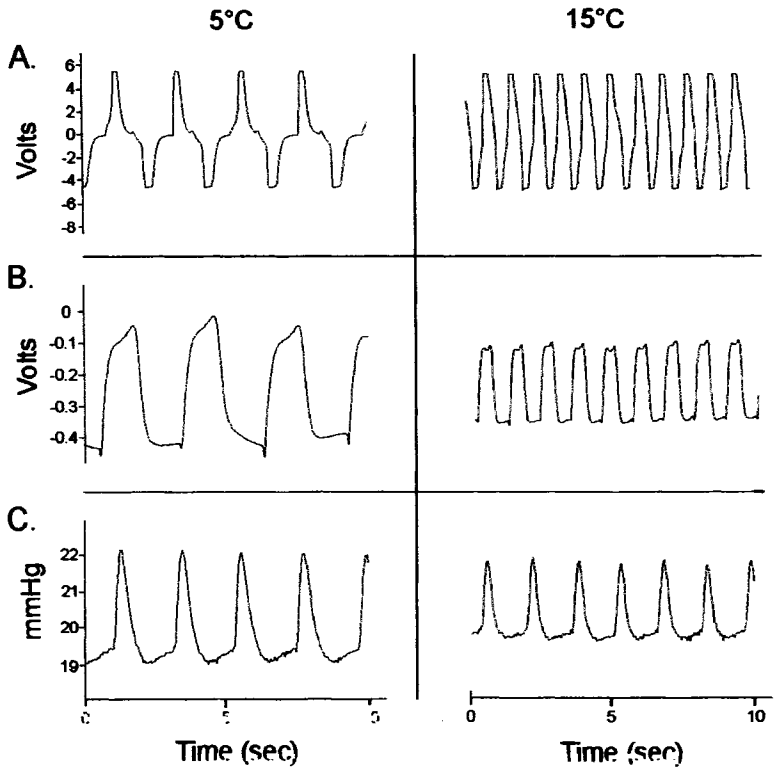


Figure 2.2. Representative recordings of heart activity obtained from three different preparations at two different temperatures: A. intact lobsters, B. lobsters with lesioned cardioregulatory nerves and C. isolated lobster hearts. The temperature at which the recording were made is indicated at the top of each column. For A and B, heart activity was recorded as a change in dynamic resistance measured by an impedance converter. For C, heart activity was recorded by monitoring the blood pressure in the sternal artery in mmHg.

(n = 7). This rate was not significantly different from the basal rate of intact lobsters at 5°C ($p = 0.69$). Isolated heart rates were also not statistically different from those of intact lobsters at 10°C ($p = 0.12$). However, at higher temperatures (15°C and 20°C), isolated heart rates were significantly lower than those observed for intact lobsters ($p < 0.005$ and $p < 0.001$ respectively). By 25°C, all but two hearts had failed completely, so averages could not be calculated. Overall, from 5°C to 20°C, there was a 71.5% increase in the rate of beating of isolated hearts (Figure 2.3), but it was not statistically significant ($p = 0.06$).

As with intact animals, Q_{10} values for isolated hearts (Table 2.1) indicate that increase of heart rate was greatest at the low end of the temperature range. Unlike intact animals however, at temperatures greater than 15°C heart rates decreased. Values obtained for the 20 - 25°C interval were eliminated from statistical analysis because most hearts failed during this step. Control hearts (n = 3) maintained at 15°C for 6 hours showed no significant changes in rate ($p = 0.99$).

Cardioregulatory nerve lesions

Representative samples of cardiac recordings from lobsters with lesioned cardioregulatory nerves are presented in Figure 2.2B. The average basal heart rate at 5°C for lobsters with lesioned cardioregulatory nerves was 18.34 ± 1.7 BPM (n = 5). This rate was not significantly different from the basal heart rate of intact lobsters at 5°C ($p = 0.14$), although it was significantly lower than the rate of excised hearts at 5°C ($p = 0.01$). A significant increase in heart rate of lobsters with lesioned cardioregulatory nerves ($p = 0.005$) was observed as the

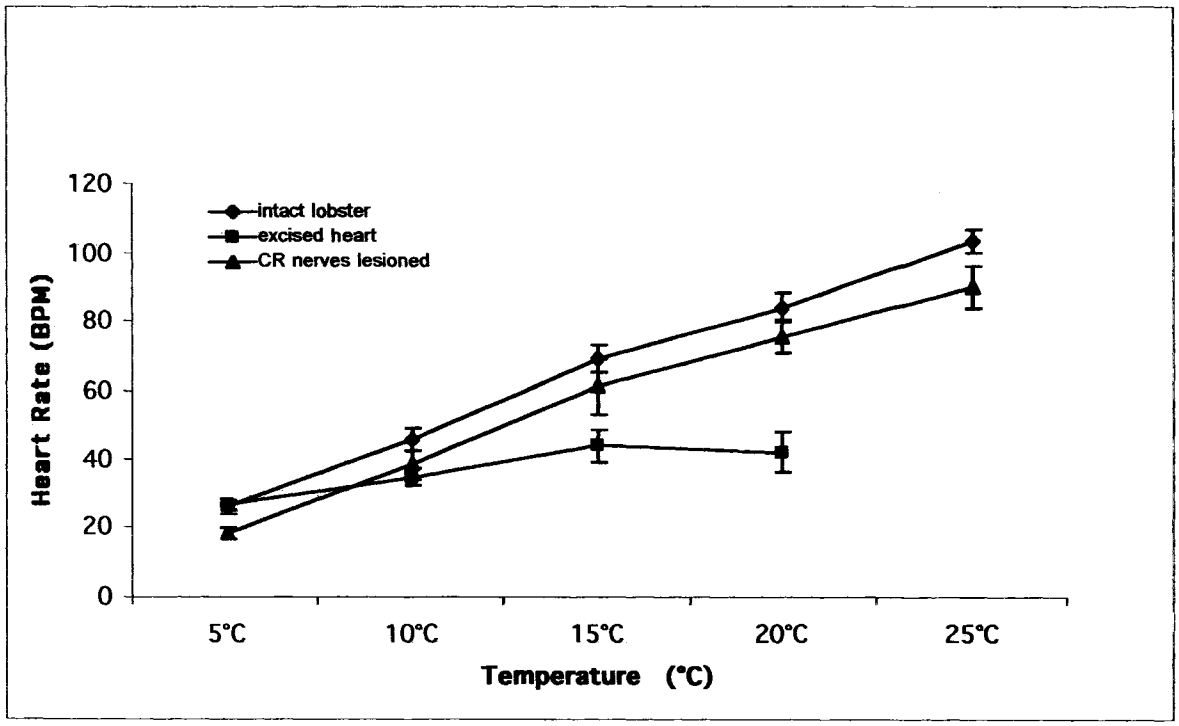


Figure 2.3. The effect of temperature on heart rates of intact lobsters (n = 25), excised lobster hearts (n = 7) and lobsters with lesioned cardioregulatory nerves (n = 5). Rates presented are averages +/- SEM.

	5-10°C	10-15°C	15-20°C	20-25°C
Intact animals (n = 25)	3.66 ± 0.4	2.60 ± 0.2	1.54 ± 0.1	1.71 ± 0.1
Isolated hearts (n = 7)	1.78 ± 0.2	1.76 ± 0.4	0.92 ± 0.1	*
CR nerve lesions (n = 5)	5.37 ± 1.3	4.30 ± 2.6	1.63 ± 0.2	1.63 ± 0.2

Table 2.1. Q_{10} values for each 5°C temperature interval for all three experimental protocols. Values are presented as avg Q_{10} ± SEM (Instat). Since all but two isolated hearts failed before 25°C (*), Q_{10} was not calculated for the 20-25°C interval for isolated hearts.

temperature was raised from 5°C to 25°C (Figure 2.3) and there was a strong correlation between temperature and rate ($r^2 = 0.84$). Overall, the slope of the regression line for lobsters with lesioned cardioregulatory nerves was not significantly different from what was observed in intact lobsters. However, the elevation of the regression line was significantly less for lobsters with lesioned cardioregulatory nerves in comparison to intact lobsters ($p < 0.05$). This is due to a lower basal rate at 5°C in animals with lesioned nerves, but a similar response of the heart to temperature. Again, Q_{10} values (Table 2.1) indicate the greatest increase in rate at the low end of the temperature range and rates continued to increase as temperature increased.

Discussion

When intact lobsters were exposed to a slow (<0.1°C/min) increase in temperature within a range that could be experienced in the field their heart rate increased in a linear manner. However, in isolated heart preparations there was no significant increase in heart rate between 5 and 20°C. The semi-isolated hearts of *Cancer magister* (DeWachter and Wilkens, 1996) also exhibited a reduced sensitivity to temperature. The slope of this increase in rate was significantly lower than that observed in intact crabs and the correlation between heart rate and temperature only existed over part of the range of experimental temperatures (4-18 °C) (DeWachter and Wilkens, 1996). Increases observed in intact animals are likely a result of exogenous inputs lacking in isolated hearts.

Isolated hearts failed completely between 20 and 25°C. This may also have been a consequence of the loss of exogenous inputs that allow for metabolic thermal compensation at high temperatures. In the crab, *Carcinus maenas*, hypoxia has been associated with a decline in beat rate in isolated hearts (Wilkens, 1993). Dissolved oxygen concentrations are affected by temperature. The solubility of oxygen decreases about 1.6% for each 1°C rise in temperature (Prosser and Brown, 1961). Over the range of temperatures examined in this study (5-25°C), dissolved oxygen (DO) concentration decreases by 32%. At 5°C, the approximate DO concentration is 12.7mg/L so at 25°C, this would decline to approximately 8.5mg/L (Clescerl *et al.*, 1965). Oxygen uptake by cells relies on a difference in the partial pressure of oxygen inside and outside the cell. This gradient is maintained by efficient aerobic metabolic pathways

within the cell, the rates of which are controlled by enzymatic activity. In the shrimp, *Farfantepenaeus paulensis*, citrate synthase activity is associated with maintaining cellular oxygen levels in response to low oxygen availability (Lemos *et al.*, 2003). Regulation of this type of enzymatic activity may be exogenous and thus lost in isolated hearts. If that were true, isolated hearts would have a reduced aerobic scope as compared to intact animals. Additionally, in the spider crab, *Maja squinado*, low oxygen availability at high temperatures has been shown to leads to the build up of metabolites like lactate and succinate, which subsequently affect performance of heart muscle cells (Frederich and Portner, 2000). Accumulation in isolated hearts could be more extreme since perfusion of tissue may not be as efficient and could contribute to the failure of the hearts.

The basal rates for isolated hearts were lower than for intact lobsters at any given temperature. Reduced basal rates in isolated hearts have also been documented in *Carcinus maenus* and *Cancer magister* (Wilkens and Mercier, 1993; Wilkens and McMahon, 1994; DeWachter and Wilkens, 1996). Cooke (1988) noted that, while the isolated cardiac ganglia of crustacean hearts consistently exhibited rhythmic bursting activity, the burst rates were often at the low end of the range reported for intact animals. Wilkens and Mercier (1993) suggested that these lower rates represented the normal cardiac burst generation in the absence of extrinsic inputs, such as stimulation by the cardioregulatory nerves (Field and Larimer, 1975; Young, 1978; Ikeya, 1984; Wilkens and Young, 1992) or by circulating neurohormones (Wilkens and McMahon, 1992).

In order to investigate the role of one such extrinsic input to the normal cardiac function, I lesioned the cardioregulatory nerves in otherwise intact lobsters. The influence of temperature on the heart rate of lobsters in which the cardioregulatory nerves had been severed was similar to that observed in intact lobsters. The cardioregulatory nerves, which provide central nervous system input to the cardiac ganglion (Alexandrowicz, 1932), are known to have at least partial extrinsic control over the cardiac functions of crustaceans (Maynard, 1953; Florey, 1960; Field and Larimer, 1975; Wilkens and Walker, 1992; Guirguis and Wilkens, 1995; O'Grady, 2001). Lesioning of these nerves has been shown to affect cardiac responses to increased activity and injected serotonin, and also to limit the ability of the heart to control stroke volume (Guirguis and Wilkens, 1995). They have also been associated with short-term responses to acute temperature change (Jury & Watson, 2000). However, the results reported in this manuscript indicate that the cardioregulatory nerves do not provide the main controlling input for the heart rate response to temperature. The results of this experiment show virtually no difference in the responses of intact animals and animals with lesioned cardioregulatory nerves.

In the absence of input from the cardioregulatory nerves, the most likely sources of neurohormones that could influence the heart rate are the pericardial organs. The pericardial organs, first identified by Alexandrowicz (1953), are neurohemal structures located in the pericardial sinus of crustaceans. It has been repeatedly demonstrated that secretions of these organs affect various aspects of crustacean cardiodynamics. Early work on the effects of pericardial organ

secretions on the heart suggested the presence of multiple substances, some inhibitory and some acceleratory (Alexandrowicz, 1953; Alexandrowicz and Carlisle, 1953, Belamarich and Terwilliger, 1966). More recent work has identified numerous components of pericardial extracts and examined their effects on heart activity. Some substances examined include serotonin (Maynard and Welsh, 1959; Cooke, 1966; Berlind and Cooke, 1970; Beltz and Kravitz, 1983; Cooke, 1988; Wilkens and McMahon, 1992), dopamine (Cooke, 1966; Cooke and Hartline, 1973; Cooke, 1988; Kuramoto and Tani, 1994), octopamine (Grega and Sherman, 1975; Evans *et al.*, 1976; Florey and Rathmayer, 1978; Battelle and Kravitz, 1978; Benson, 1984; Cooke, 1988; Kuramoto and Tani, 1994), proctolin (Kingan and Titmus, 1983; Schwartz *et al.*, 1984; Siwicki *et al.*, 1987; Cooke, 1988; Wilkens and McMahon, 1992; Wilkens and Mercier, 1993) and crustacean cardioactive peptide (CCAP) (Dircksen and Keller, 1988; Wilkens and McMahon, 1992; Wilkens and Mercier, 1993). The effects of each of these components, as well as the overall composition of pericardial extract, varies significantly between crustacean species but, in general, pericardial secretions act to enhance heart activity by increasing the amplitude and frequency of contractions (Florey and Rathmeyer, 1978; Cooke, 1988; Wilkens and McMahon, 1992; Wilkens and Mercier, 1993). These effects are often long lasting, and thus it has been suggested that some or all of the components of pericardial secretions may be acting as hormones (Florey and Rathmeyer, 1978; Beltz and Kravitz, 1983; Schwarz *et al.*, 1984; Cooke, 1988; Wilkens and Mercier, 1993). It has been suggested that hormones from the pericardial organs could cause

changes in cardiodynamics in response to long-term changes in metabolic demands or environmental conditions (Wilkens, 1987; Wilkens and Mercier, 1993).

Serotonin, octopamine and a substance similar to proctolin have been localized in the pericardial organs of the lobster (Beltz and Kravitz, 1983). All of these substances have been shown to affect cardiac output in lobsters when applied to isolated hearts. Both serotonin and octopamine cause increases in frequency and amplitude of contraction, but much larger quantities of octopamine are required to achieve the same result. Application of proctolin elicits a change in amplitude but shows no effect on frequency of contraction (Wilkens, 1987). Based on the available data, the relationship between serotonin and temperature should be investigated.

In conclusion, our results in lobsters, taken together with previous results in other crustacean species, suggest that temperature-dependent changes in heart rate are not due to the direct influence of temperature on the heart muscle and/or the cardiac ganglion. Rather, in intact lobsters, increased temperatures must be imposing metabolic demands, which are sensed by the central nervous system. This metabolic increase results in the release of cardioactive modulatory substances from a source other than the cardioregulatory nerves. The pericardial organs, which also influence heart rate through the release of neurohormones, are the likely source of these modulatory substances, and serotonin is a likely candidate. My results support this idea in that I observed no significant differences in the responses of animals with lesioned cardioregulatory nerves

and intact animals, both of which would have regulatory input from the pericardial organs. The isolated hearts, however, have no pericardial influences and they exhibited no significant increase in rate in response to increased temperature. The identity of the neurohormones involved needs to be further investigated.

CHAPTER III

TEMPERATURE AVOIDANCE BEHAVIOR IN THE AMERICAN LOBSTER, *HOMARUS AMERICANUS*.

Introduction

The American lobster (*Homarus americanus*) is widely distributed throughout the northwestern Atlantic Ocean. Their distribution extends from the coast offshore to the edge of the continental slope, and inland into estuarine habitats. Throughout this range, the lobster is exposed to a wide range of ambient temperatures from near 0°C in coastal or estuarine environments during the winter to as high as 27°C in estuarine habitats during summer. Both of these values approach the lethal tolerance limits of the species (McLeese, 1956).

It is generally accepted that temperature exerts a strong influence on the behavior of lobsters (Herrnkind, 1980; Aiken and Waddy, 1986; Factor 1995). For example, it may play a role in controlling the direction and/or magnitude of large- and small-scale seasonal movements of lobsters (Saila and Flowers, 1968; Cooper and Uzmann, 1971; Dennert and Van Maren, 1974; Kanciruk and Hernnkind, 1978; Munro and Therriault, 1983; Ennis 1984; Karnofsky *et al.*, 1989; Lawton and Lavalli, 1995; Watson *et al.*, 1999). Higher temperatures have also been linked to increased catch (Paloheimo, 1963; Flowers and Saila,

1972; Dow, 1977; Dow, 1978; Fogarty, 1988; Campbell *et al.*, 1991; Hudon, 1994; Koeller, 1999) which may be the result of increased activity levels, leading to more frequent encounters with traps and fishing gear. However, all of these studies are merely showing correlations between water temperature and activity while many other abiotic factors may also be influencing the locomotory behaviors.

It has been previously suggested that ectothermic animals express a preference for thermal niches in which their metabolism is optimal or most efficient (Beitinger and Fitzpatrick, 1979; Magnuson *et al.*, 1979; Reynolds and Casterlin, 1979; Hutchinson and Maness, 1979; Kiviuori, 1980; Mundahl and Benton, 1990; Taylor, 1990). This refers to a range of temperatures where metabolic rates remain relatively low, while growth rates are maximized and the animal remains within its aerobic scope. Moving out of this range can result in thermal metabolic compensation, when enzymatic activity increases to compensate for the temperature change and metabolic rate increases (Hochachka and Somero, 1984; Lemos *et al.*, 2003). Avoidance behaviors may occur to minimize increases in metabolic rates.

The preferred and avoided ranges are not absolute, as they is known to shift with acclimation. In the decapod crustaceans, crayfish exhibit both temperature avoidance and preference behaviors and these responses are affected by acclimation history (Espina *et al.*, 1993; Kivivuori, 1994). In the American lobster, Reynolds and Casterlin (1979) demonstrated that, when allowed to move freely within a temperature gradient that ranged from 0-50°C,

adult lobsters selected the relatively narrow range of 15-21°C the majority of the time and exhibited a distinct thermal preference for ~16°C. No effects of acclimation history were addressed. It is stated in the paper that since the lobsters exhibited the same thermal preference over a time span of 6 days, it was independent of their previous acclimation experience.

Crossin *et al.* (1998) expanded on this work by evaluating preferred and avoided temperatures for both summer- and winter-acclimated lobsters. The gradient used for preference experiments had a different range (12-22°C) and yet they found a final thermal preference for summer lobsters that was similar to the 16°C value by Reynolds and Casterlin (1979). Crossin *et al.* (1998) also evaluated avoidance, both in the gradient and in an avoidance trough, in which animals were forced to leave a shelter by slowly heating the water until they found it aversive. Within the gradient, the lobsters avoided the highest and lowest temperatures (<13°C and >19°C). This behavior suggests they may be demonstrating orthokinesis (Fraenkel and Gunn, 1961), meaning they do not actively select a preferred temperature but rather avoid the extremes. Although this result does not conclusively demonstrate the mechanism for behavioral responses to temperature in the lobster, it eliminates the possibility that avoidance is simply an artifact of increased activity at higher temperatures because lobsters in the study avoided low temperatures as well, when locomotion would be expected to decline.

In the avoidance trough experiments, summer lobsters avoided temperatures greater than 23.5°C, which was about 8°C warmer than ambient.

However, winter lobsters could not be forced to exhibit avoidance when exposed to the same 8°C temperature increase. Crossin *et al.* (1998) suggest that this lack of avoidance behavior observed in winter lobsters indicates that lobsters, regardless of acclimation history, find temperatures greater than 23.5°C aversive. Since the 8°C temperature increase did not expose winter lobsters temperatures above this threshold, they would not be expected to exhibit avoidance. This conclusion is unlikely based on previous research showing effects of acclimation on behavioral thermoregulation in other decapod species (Espina *et al.*, 1993; Kivivuori, 1994). Individuals with different acclimation histories should have significantly different thermal tolerances.

Other factors that may play a role in determining the scope of behavioral thermoregulation in lobsters are size and sex. In the Great Bay estuary system, lobsters undergo a seasonal migration, entering the estuary in late spring or early summer and leaving in the late summer and fall (Watson *et al.*, 1999). However, there seems to some sex-based differential in the scope of this migration. Significantly more adult males are found in the upper reaches of the estuary (Howell *et al.*, 1999). This could suggest that males enter the estuary earlier in the spring and therefore are able to travel farther. Alternatively, it could indicate that males are tolerant of higher temperatures or other abiotic factors that are more variable in the upper estuary. Interestingly, the skewed sex ratio only exists for adult lobsters. For smaller sizes classes, the ratio of males to females is equal. This suggested that size or life history phase may also contribute to the scope of migratory activities.

The experiment presented in this paper expands on the work presented in Crossin *et al.* (1998). Avoidance experiments are repeated with a wider range of sizes (47-92mm CL) and higher maximum temperatures to elicit avoidance. The goal of these trials was to quantify the contributions of sex, size and season to the behavioral thermal tolerance limits of lobsters. The hypothesis is that all lobsters, regardless of acclimation history (or in the case, season), will exhibit avoidance after the same relative temperature increase. Consequently, lobsters acclimated to summer temperatures will show a significantly different absolute avoided temperature than those acclimated to winter temperatures.

Methods

Animals

Lobsters of both sexes were obtained using both standard and ventless lobster traps located near the University of New Hampshire (UNH) Coastal Marine Laboratory in Newcastle, NH. Immediately after capture, lobsters were tagged and sexed, and carapace length (CL) was measured. All lobsters used in these experiments were maintained in 1m diameter holding tanks with continuous ambient water flow and additional aeration provided by airstones. Lobsters were fed baitfish weekly.

A total of 60 avoidance trials were completed. Of these trials, the initial 43 were completed between the months of June and September during 1998 and 1999 and were classified as "summer" trials. The remaining 17 were completed between the months of January and April of 2000 and were classified as "winter" trials. Summer trials involved lobsters in all five size classes, while winter trials only involved lobsters in the three largest classes (65-75, 75-85 and 85-95 mm CL). There were 21 female and 22 male lobsters used in the summer trials and they ranged in size from 47-90mm CL. There were 4 female and 13 male lobsters used in the winter trials that ranged in size from 83-92 mm CL.

Avoidance Tank Design

Avoidance experiments were performed in an 11'10" x 7.5" trough tank (Figure 3.1). A water inflow pipe with a shutoff valve was located at the head of

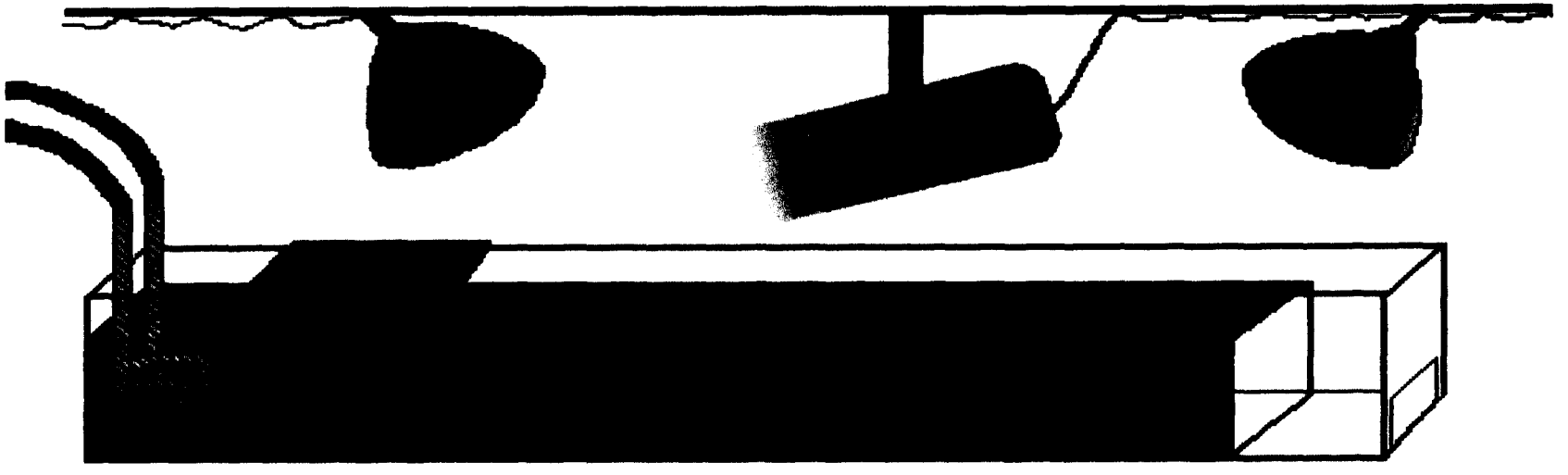


Figure 3.1. Trough tank used for temperature avoidance experiments. An inflow pipe was positioned at the head of the tank near the heat transfer coil and water flowed out over the Plexiglas barrier at the opposite end of the tank.

the tank. Approximately 2'8" from the end of the tank, a Plexiglas wall was installed to maintain a water level of 5 inches. Beyond that wall, water flowed freely out of the tank through a drain. An 8-inch section at the head of the tank contained an aluminum heat exchange coil, which was connected to a recirculating temperature control unit (model 1162, VWR Scientific, Niles, IL). A wire mesh barrier prevented the lobster from entering this head section. Two airstones were placed within the head section to prevent stratification.

A section of 3/4" plywood was placed over the trough to cover the head section plus an additional 1' section of the tank to create a shelter for the lobster. A 40W light was suspended at each end of the tank to encourage the lobster to remain in the shaded shelter area. An additional airstone was placed half way down the length of the tank to reduce stratification. To monitor the movements of the lobster in and out of the shelter area, a low-light camera was suspended over the tank. The camera was connected to a monitor in an adjacent room where observations took place. The walls of the entire tank were covered 1" polystyrene foam for insulation and surrounded with a black plastic drape during all acclimation times and experiments to limit disturbance.

Temperature Avoidance Trials

Methods for avoidance trials were similar to those described in Crossin *et al.* (1998) and were identical for summer and winter trials. Prior to being placed in the tank, the sex and carapace length of the lobster were recorded and a Tidbit submersible temperature data logger (Onset, Bourne, MA, USA)

was attached to the dorsal carapace using cyanoacrylate gel and Velcro. The lobster was then released into the center of the trough tank. For all trials, lobsters were allowed to acclimate to the tank overnight.

Prior to the start of the avoidance trial on the following day, it was determined whether or not the lobster was in the shelter at the head of the trough tank. If the lobster was not in the shelter, the trial was abandoned and a new lobster was prepared and placed in the tank. If the lobster was in the shelter, the avoidance trial proceeded.

At the start of a trial, the inflow to the trough tank was shut off. The lobster was observed remotely for 20 minutes to determine if the change in flow caused them to abandon the shelter. If the lobster left the shelter during this first observation period, the trial was abandoned. If the lobster remained in the shelter for the first observation period, then the recirculating temperature control unit was turned on to heat the water in the shelter. The temperature control unit was set to 70°C, which resulted in an average rate of temperature change within the shelter of 0.2± 0.01°C/min (avg. ± SEM). The lobster was observed remotely and all visible movements were noted until it exited the shelter, which marked the end of the trial. The time of exit was then noted.

Data Analysis

Upon completion of a trial, the datalogger was removed from the lobster and downloaded using Boxcar Pro PPC (v.3.5d2, Onset) in order to obtain a record of temperatures experienced by the lobster (see Figure 3.2 for an

sample). The data from the logger were correlated with notes taken on movements during the trial. Using this method, both the actual temperature of avoidance and the amount of temperature change required to elicit avoidance were calculated for each lobster. Average avoidance temperature and average change in temperature to elicit avoidance were calculated using Instat and are presented ± SEM.

For the purpose of analysis, all animals were classified into 10 mm CL size classes (45-55, 55-65, 65-75, 75-85, 85-95mm). The data were analyzed using a multi-factor ANOVA (GLM: Systat v.9.0, Systat Software Inc., Richmond, CA, USA) with blocking by size category, sex and season to determine the contribution of each of these variables.

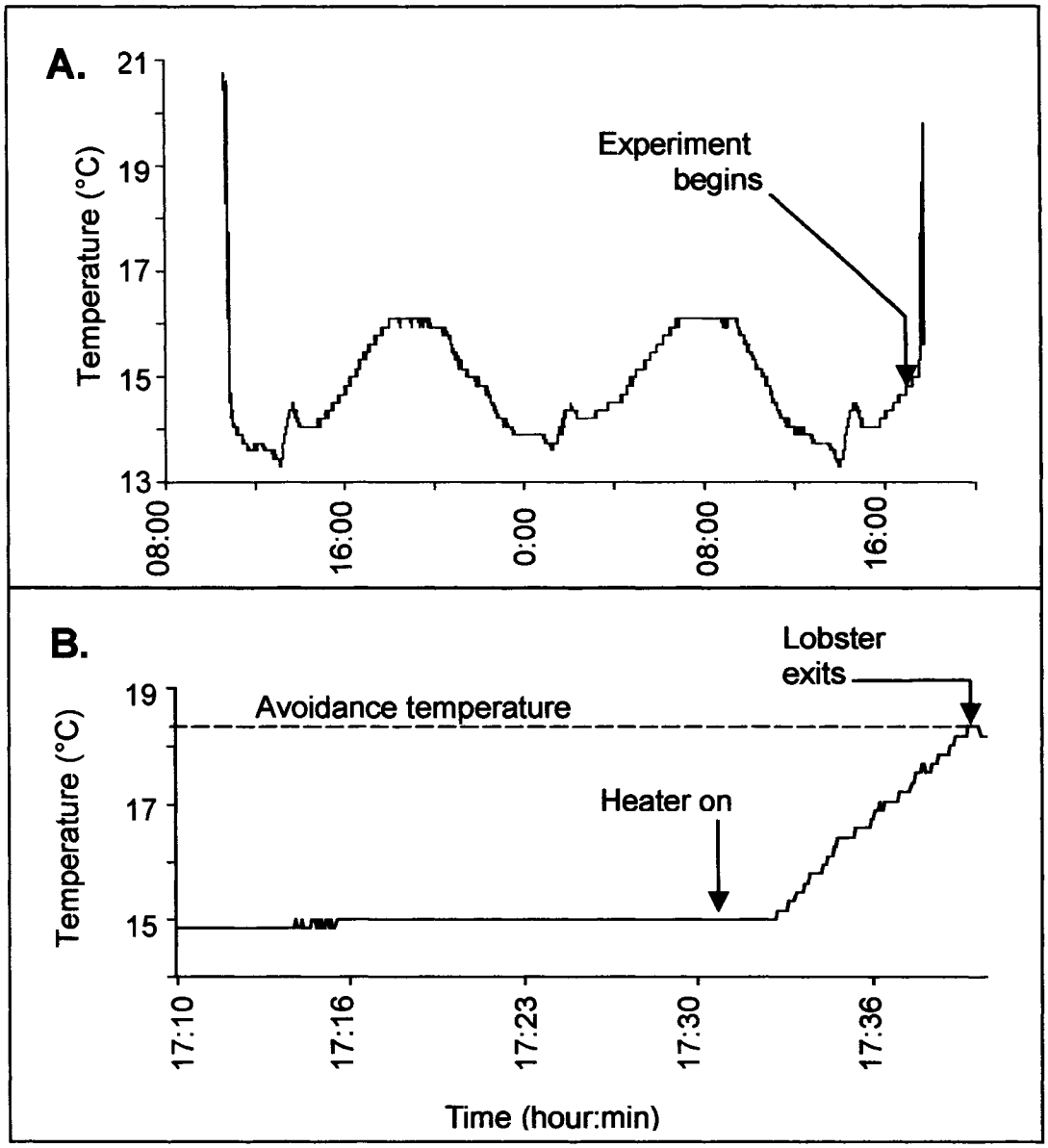


Figure 3.2. A. Sample output voltage from a Tidbit temperature datalogger (Onset) over the course of one complete avoidance run, including the acclimation day. B. Close up of the Tidbit output for the period of the experimental trial. The inflow to the tank was shut off at 17:10 and the heater was turned on at 17:31. The average rate of temperature increase during heating for all trials was 0.167+/- 0.01 °C/min. For this trial, the lobster exhibited avoidance at 17.40 when the temperature of the shelter reached 18.33°C.

Results

Summer Trials

The average avoidance temperature for all lobsters was 22.3 +/- 0.5°C. A multi-factor ANOVA revealed a significant effect of sex ($p = 0.05$) on avoided temperature but no significant effect of size category ($p = 0.07$) and no interaction effects ($p = 0.33$). Males had a significantly higher average avoidance temperature (23.1 ± 0.7°C, n = 22) than females (21.9 ± 0.9°C, n = 21) (Figure 3.3). The average change in temperature required to elicit an avoidance response was 7.5 +/- 0.6°C for all summer trials, with no effects of sex ($p = 0.19$) or size category ($p = 0.20$).

Winter Trials

The average avoidance temperature for all winter trials was 14.3 +/- 1.1°C. Contrary to what was found for summer animals, sex had no significant effect on average avoidance temperature for winter trials ($p = 0.45$). There was a trend for higher avoidance temperatures in males, but it was not significant, most likely due to the low number (n = 4) of females and the high variability they expressed.

However, there was a significant difference in avoided temperature between size classes ($p = 0.01$) for winter trials (Figure 3.4). Since there was only one lobster in the 65-75 mm CL size class, it could not be included in this analysis. Lobsters in the 85-95 mm CL size class (n = 7) exhibited a significantly higher avoided temperature than those in the 75-85 mm CL size

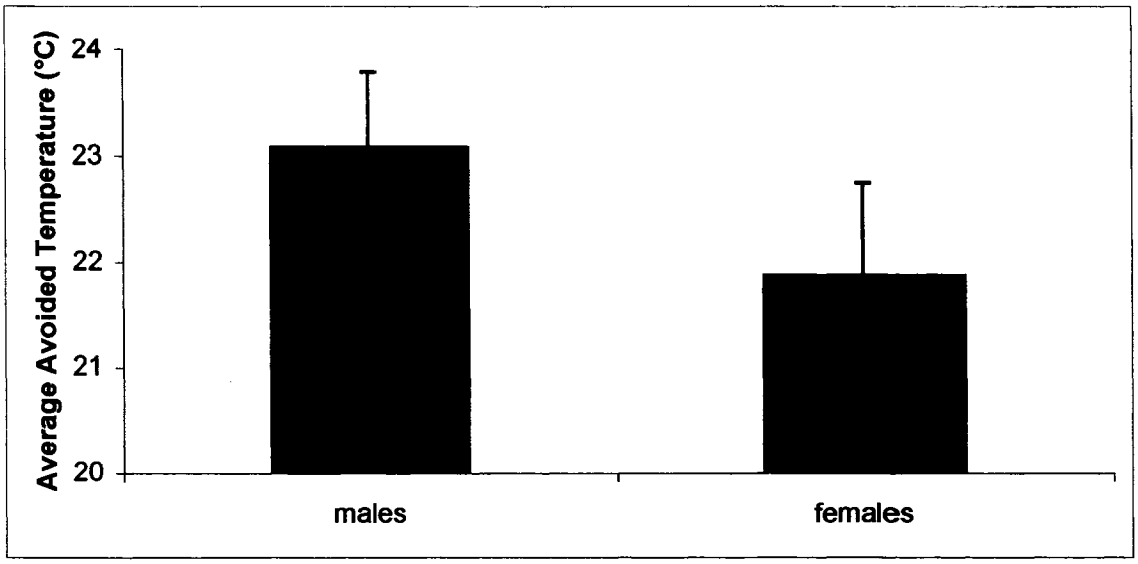


Figure 3.3. Comparison of average avoided temperature between males and females for summer trials. Males (23.09 +/- 0.7°C, n = 22) exhibited a significantly higher average avoided temperature than females (21.88 +/- 0.9°C, n = 21) (p=0.05).

class (n = 9) (Figure 3.4). The average change in temperature required to elicit an avoidance response was 9.4 +/- 1.1°C for all winter trials. Similar to summer trials, there was no effect of sex or size category on this result.

Seasonal comparisons

Multi-factor ANOVA was repeated after completion of all trials to evaluate inter-season variability. There was a highly significant effect of season on avoided temperature ($p < 0.001$), with winter animals exhibiting avoidance behaviors at much lower temperatures than summer animals (Figure 3.5A). There was no significant effect of sex or size on these results.

There were no seasonal, sex-based or size-based effects on average change in temperature required to elicit an avoidance response when comparing summer and winter trials (Figure 3.5B).

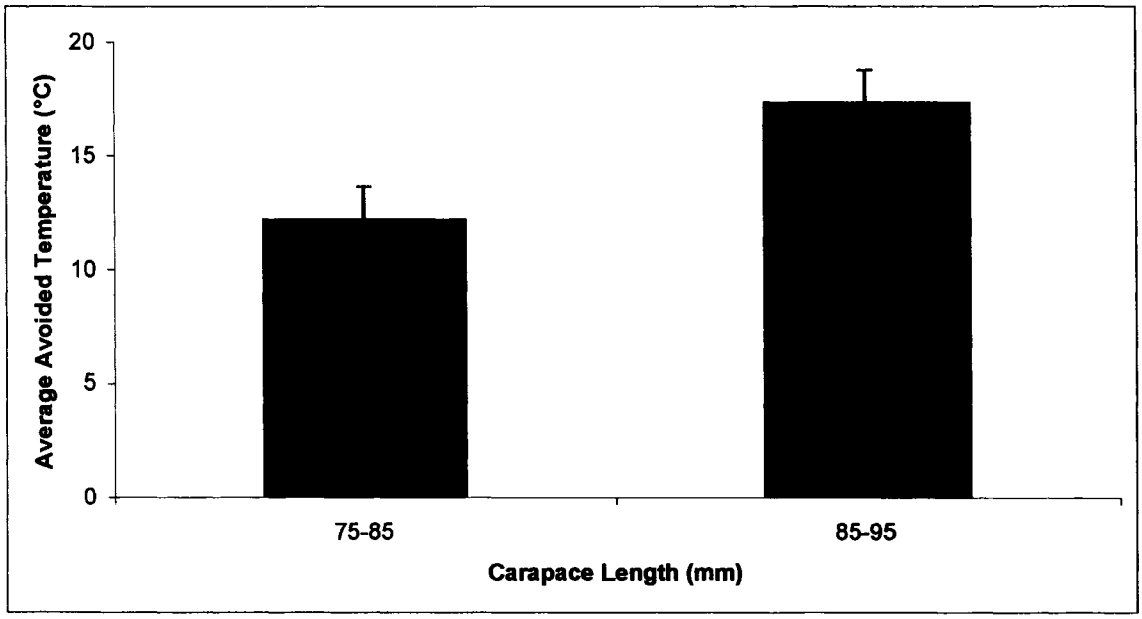
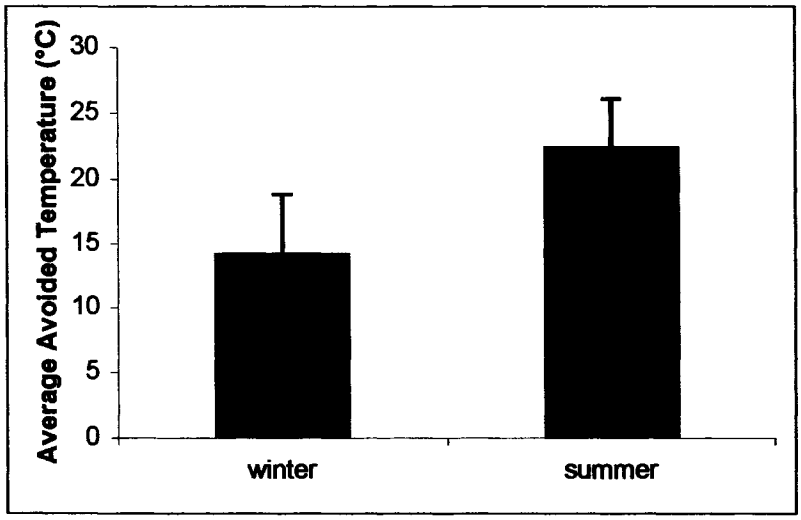


Figure 3.4. Comparison of average avoided temperatures between lobsters in two different size classes for winter trials. The larger size class exhibited a significantly higher average avoided temperature (17.31 +/- 1.5°C, n = 7) as compared to the smaller size class (12.2 +/- 1.4°C, n = 9) (p=0.01).

A.



B.

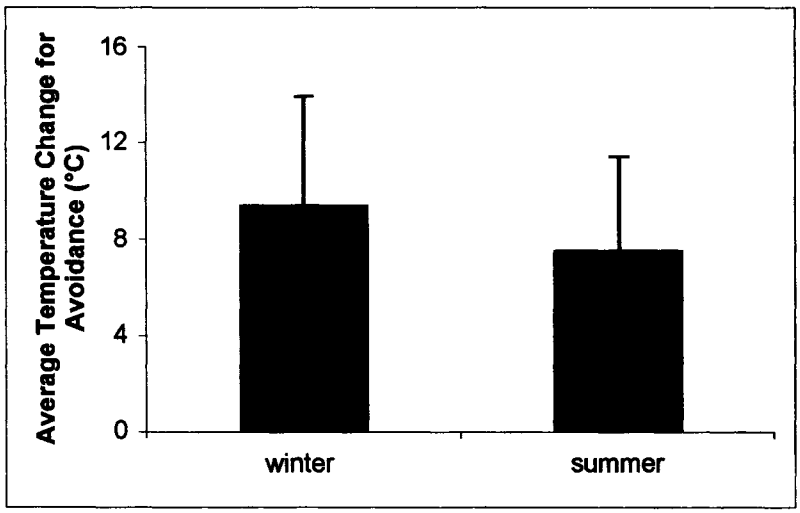


Figure 3.5. A. Seasonal comparison of average avoided temperatures. Winter lobsters (n = 17) exhibited avoidance at a significantly lower temperature (14.3 +/- 4.7°C) than summer lobsters (22.5 +/- 3.7°C, n = 43) ($p < 0.001$). B. Seasonal comparison of temperature change required to elicit avoidance behaviors. There is no significant effect of season.

Discussion

The seasonal movements of the American lobster into and out of Great Bay estuary are likely to be affected by a variety of abiotic factors. The results from this study indicate that temperature may be influencing these movements in a way that varies seasonally. Sex-based differences in avoidance were only observed under summer conditions and not for winter animals. This result correlates well with the annual distributions observed in the Great Bay estuary system, in which lobsters all exhibit an offshore distribution during the winter, but males travel farther into the estuary and into warmer waters than females during the summer (Howell *et al.*, 1999).

In contrast, size was only shown to have a significant effect on avoided temperature during the winter. This is more difficult to correlate to observed differences in distribution. There is clearly a size-based difference in distribution during the summer months based on the equal distributions of male and female juveniles in the estuary as compared to the unequal distribution of adults (Howell *et al.*, 1999). However, the observed distribution could be based on abiotic factors other than temperature.

The results of this experiment support the hypothesis that both summer- and winter-acclimated lobsters will exhibit avoidance after the same relative temperature increase. There was no significant difference between the change in temperature required to elicit avoidance in summer vs. winter lobsters. This result is contrary to what was found by Crossin *et al.* (1998) in a similar experiment, in which winter-acclimated lobsters could not be induced to exhibit

an avoidance response to warmed water. This is likely due to the temperature ranges used in the experiments. In the previous experiment, they found that summer lobsters exhibited avoidance after an 8.0°C increase. The highest temperature used to elicit avoidance in winter trials was 12.3°C, which was exactly 8°C higher than ambient. In the present experiment, summer-acclimated lobsters exhibited avoidance after a similar average increase in temperature (7.53 ± 3.9°C) and winter-acclimated lobsters required a slightly, but not significantly, higher increase (9.38 ± 4.5°C). So, the maximum temperature increase used in the winter trials of the Crossin *et al.* (1998) study was likely to have been slightly below what was required to elicit avoidance. In a previous study with crayfish (Espina *et al.*, 1993), a similar result was obtained in regards to the relationship of change in temperature required to elicit avoidance between warm and cool-acclimated animals. Espina *et al.* (1993) found that animals acclimated to a higher temperature exhibited a lower percent change before avoidance than animals acclimated to a lower temperature. This may represent the difference between entering and leaving a range of temperatures that could be beneficial for metabolic processes like growth or reproduction.

It was clear from the results of the present study that the absolute avoidance temperature was significantly lower for winter-acclimated animals (14.26 ± 4.7°C) than for summer-acclimated animals (22.5 ± 3.7°C). This result supports what has been found previously in crayfish (Espina *et al.*, 1993; Kiviuori, 1994). Most studies that address preferred temperatures or optimal

metabolic functioning of crustaceans identify a range of approximately 5°C. For example, Kivivouri (1980) determined that crayfish righting behavior is most efficient between 15-20°C. Reynolds and Casterlin (1979) found that the range of thermal preference in a gradient for the American lobster was approximately 15-21°C. Over that same range, lobster locomotion is independent of temperature changes (McLeese and Wilder, 1958; Reynolds and Casterlin, 1979), which could be suggestive of a behavioral alteration resulting in maintenance of position in the favorable habitat.

If optimal ranges are only 5°C wide, a change in temperature of approximately 8°C would always result in shifting the animal out of its preferred range. If metabolic efficiency is as closely associated with the preferred range as has been suggested (Beitinger and Fitzpatrick, 1979; Magnuson *et al.*, 1979; Reynolds and Casterlin, 1979; Hutchinson and Maness, 1979; Kiviuori, 1980; Mundahl and Benton, 1990; Taylor, 1990), and movement out of the range of preferred temperatures results in elevated metabolic rate due to increased enzymatic activity (Lemos *et al.*, 2003), then such changes could be very energetically costly. Avoidance could be initiated as a means of returning the animal to its thermal optima.

Recent evidence suggested that metabolic thermal optima shift with adaptation to new ambient temperature conditions and that undergoing this shift has associated energetic costs (Claireaux *et al.*, 1995), even if there are also benefits associated with the new thermal regime. This could explain why winter lobsters exhibited avoidance at temperatures below what is suggested

to be their species-specific optimum temperature range (Reynolds and Casterlin, 1979; Crossin *et al.*, 1998). If the physiological processes within the animals were adapted to run most efficiently at winter ambient temperatures and those temperatures were increased enough to move the animal out of its normal tolerance range, then the animal should exhibit avoidance. Winter animals exhibited avoidance at 14.26°C, which is less than the optimal temperatures from previous studies.

In summary, this research showed that the same relative ambient temperature change (rather than a specific absolute temperature) elicits avoidance behaviors in lobsters regardless of acclimation season. Males in general are more tolerant of high temperatures than females regardless of size or season. Both of the conclusions help to explain the annual distribution pattern observed in the Great Bay estuary. To further elucidate the influences of temperature on this distribution, it will be necessary to establish avoidance thresholds to declining temperature as well. This is relevant to the triggering of migration out of the estuary during late summer and fall. Also, it would be beneficial to continue monitoring seasonal distribution in the estuary as well as seasonal variations in abiotic variables. Long-term population statistics combined with data on environmental conditions have been used previously to create predictive models of crustacean abundance and distribution (Atrill *et al.*, 1999). The creation of such a model for the population of lobsters in the Great Bay estuary system would not only be scientifically significant but also commercially beneficial for the management of the fishery.

CHAPTER IV

THE EFFECTS OF TEMPERATURE, SEX AND SIZE ON LOCOMOTORY ACTIVITY IN JUVENILE LOBSTERS, *HOMARUS AMERICANUS*.

Introduction

Throughout their range in the northwestern Atlantic Ocean, the American lobster (*Homarus americanus*) is exposed to a wide range of ambient temperatures. For lobsters that utilize estuarine environments such as the Great Bay estuary in New Hampshire, temperatures can vary from near 0°C during winter to 27°C or greater in the summer months. No other abiotic variable, other than light level, varies this greatly on a regular basis. Consequently, temperature has long been considered as a possible controlling factor in many aspects of lobster physiology and behavior, specifically regarding activity patterns (McLeese and Wilder, 1958; Herrnkind, 1980; Aiken and Waddy, 1986; Factor 1995).

The effect of temperature on lobster locomotor activity was first addressed by McLeese and Wilder (1958). They found that, over a wide range of temperatures, the walking rate of lobsters in response to exposure to a bright light source generally increased with temperature. This relationship has since been used to support the argument that temperature is the main influence on both small- and large-scale seasonal movements of lobsters (Saila and Flowers, 1968; Cooper and Uzmann, 1971; Dennert and Van Maren, 1974; Kanciruk and

Hernnkind, 1978; Munro and Therriault, 1983; Ennis 1984; Karnofsky *et al.*, 1989; Lawton and Lavalli, 1995) as well as on catchability of lobsters (McLeese and Wilder, 1958; Koeller, 1999). However, upon closer examination, the results from McLeese and Wilder (1958) show a linear relationship between temperature and walking rate only existed at the extremes of the temperatures examined (2-10°C and 20-25°C). In the intermediate temperature range, which extended from 10-20°C, activity levels were constant regardless of temperature. In natural conditions, this temperature range represents the normal condition for coastal and estuarine population of lobsters for much of the year. If activity levels are truly independent of temperature over this range, the influence of temperature on seasonal movements and catchability must be called into question. However, it is unclear whether this apparent zone of temperature independence exists in lobsters in the field or those exposed to seasonally-appropriate ambient temperature changes. Additionally, McLeese and Wilder (1958) only examined activity in adult lobsters and, although they used lobsters of both sexes in their experiments, they did not consider sex-based differences. In order to elucidate the role of temperature in controlling population dynamics such as seasonal movements and catchability, it would be necessary to follow parameters more similar to the natural environment.

The relationship between temperature and locomotor activity in lobsters was further examined by Jury in 1999. In this study, spontaneous activity (as measured by distance traveled per day) was monitored in lobsters exposed to seasonally-appropriate temperatures. Lobsters of both sexes were used in this

experiment, permitting sex-based comparisons. However, all lobsters were adult (81-92 mm) so ontogenic changes were not observed. The overall relationship between temperature and activity was found to be non-linear. Instead, Jury suggested the existence of two distinct "activity states". Below 10°C, activity levels were low and relatively constant (0-50 m/day) while above 10°C, activity levels increased but also became more variable (near 0 – near 200 m/day). No sex-based differences were observed, suggesting that males and females are influenced in a similar way by temperature. The results of this study support the belief that temperature could be influencing seasonal migrations and catchability as ambient temperatures shift lobsters from a low activity state to a high activity state.

The experiment presented in this chapter expands on the work by Jury (1999) by considering the effects of seasonally-appropriate ambient temperatures on juvenile lobsters of both sexes. By including a larger range of sizes, it may be possible to elucidate ontogenic changes in the response to temperature and to determine if the effects of temperature on locomotor activity in lobsters are universal across size classes and life history categories.

Methods

Animals

Adult and juvenile lobsters of both sexes, between 35 and 68 mm carapace length (CL), were obtained using standard and ventless lobster traps. These traps were located near the University of New Hampshire (UNH) Coastal Marine Laboratory (CML) in Newcastle, NH. All lobsters were maintained in flow-through seawater tanks at the CML. Two methods were used to monitor activity of the lobsters: magnetic reed switch arrays and time-lapse video.

Activity Monitoring Using Magnetic Reed Switches

In order to monitor lobster activity remotely, a "racetrack" system was used (Figure 4.1a). It consisted of two concentric rings of plastic coated wire mesh (2 cm x 1 cm). The dimensions of the rings were 61 cm and 20.5 cm respectively. The resulting dimensions of the track in which the lobster was able to freely move were 1.29 m x 20.5 cm x 30.5 cm (L x W x H). Two shelters composed of bisected PVC were positioned on opposite sides of the track (15.5 cm x 15.5 cm, L x H). The shelters extended across the entire width of the track.

On the under side of each shelter, an array of 5 magnetic reed switches was mounted. These sensors respond to changes in magnetic field. Lobsters used in these experiments had small magnets attached to their dorsal carapace using cyanoacrylate gel and tape. When a lobster passed under a set of magnetic reed switches, it resulted in a change in output voltage from that set of

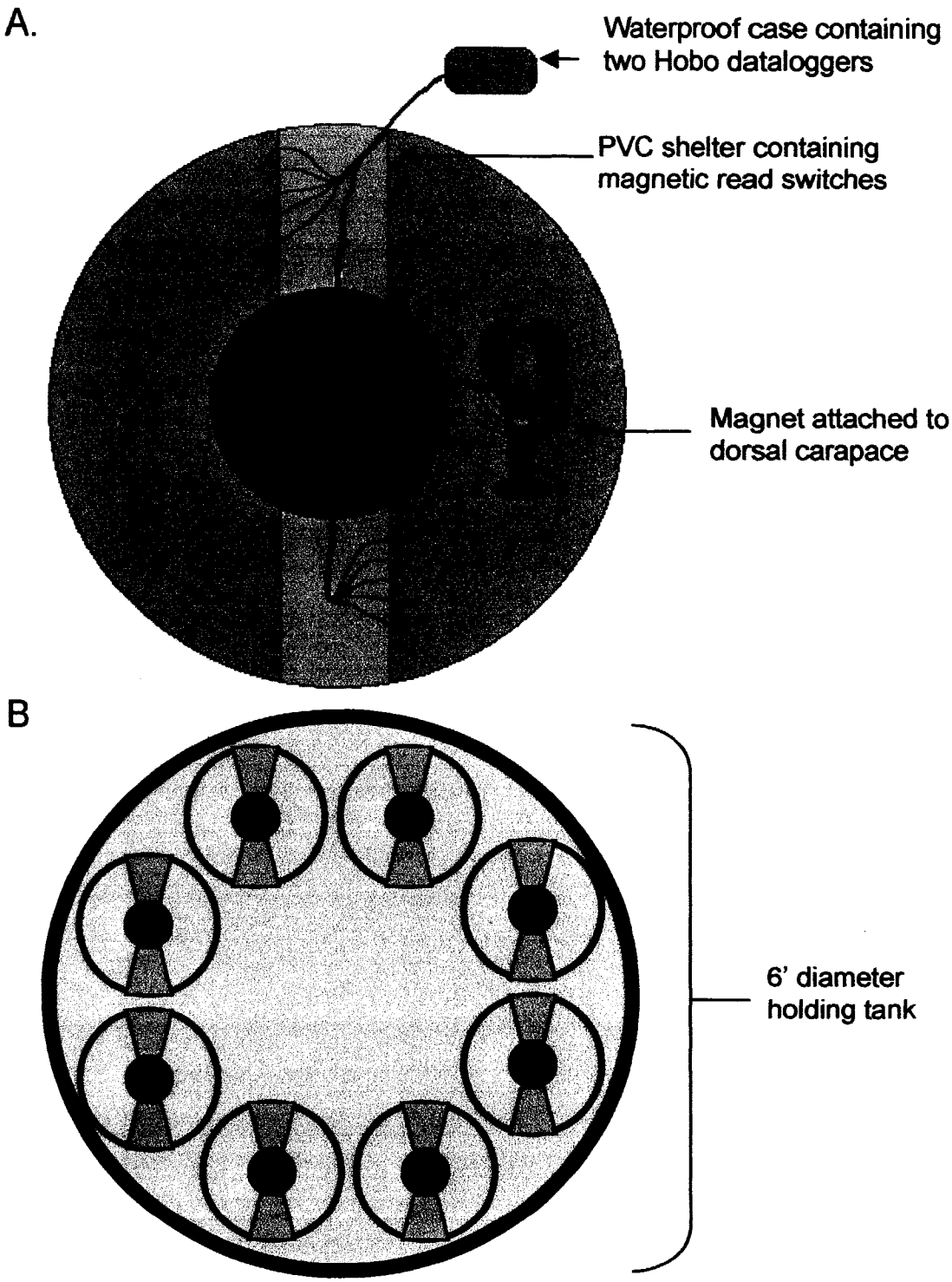


Figure 4.1. A. Activity racetrack with magnetic read switch arrays. B. Smaller activity racetracks positioned in a larger 6' holding tank. Small racetracks are not equipped with reed switches. Rather, a low-light camera and infra-red array are positioned above the tank.

switches. The output was monitored by two dataloggers (HOBO 4 channel external, Onset Computer, Bourne, MA, USA), each capable of recording approximately 13 hours of data. These dataloggers were connected in series and one was always set to start recording with a 12-hour delay. This allowed collection of 24 hours of uninterrupted data.

Dataloggers were downloaded daily during this experiment to allow continuous monitoring of reed switch output. When either set of magnetic reed switches was triggered, it appeared as a change in voltage on the datalogger recording. In each track, one shelter was wired directly to the datalogger. A resistor was added to the wiring of the second shelter so that the output voltage would be reduced. This allowed us to determine which set of reed switches had been triggered.

Two activity racetracks were used in this experiment. Each racetrack was set up in a separate 3' holding tank with flow-through seawater circulation and a water depth of approximately 32 cm. The holding tanks were covered with black plastic to minimize disturbance and to allow control of the light cycles throughout the experiment. A light fixture with a 40W bulb was suspended over each tank. These lights were controlled by programmable timers set for a 7-day cycle. The timers were set to provide an ~14/10 light/dark schedule. Two dataloggers were placed in each holding tank, one to record light levels (HOBO Light Intensity, Onset Computer) and the other to record water temperature (HOBO Temp, Onset Computer) throughout each trial.

On the first day of an activity trial, a lobster was placed in the activity racetrack. After 24 hours, voltage output dataloggers were downloaded (Boxcar Pro PPC, v 3.5d2, Onset Computer) and relaunched. At the end of the second 24 hours, voltage output dataloggers were again downloaded and the lobster was removed from the track to allow a new trial to begin.

Activity Monitoring Using Video

After several attempts to use the racetrack system described above to monitor activity of very small lobsters (<45 mm CL), it was determined that the system was ineffective for lobsters of this size. Only very small magnets could be attached to these small lobsters and these magnets did not consistently trigger the reed switches. Subsequently, a video monitoring system was devised. Although initially designed for use with small lobsters, this system proved to be very effective and was eventually employed with larger lobsters as well.

The racetracks used in this protocol were similar to, but smaller than, those described previously (OD 46 cm, ID 18 cm, Figure 4.1b). In all video monitoring trials, shelters were constructed of plastic coated wire mesh that had been bent to fit within the track. Black plastic was wrapped around the wire mesh of the shelters to provide opacity. Black plastic was also wrapped on the sides of the track to minimize visual disturbances between neighboring tracks. On tracks used for the smallest lobsters (35-40 mm CL), the bottom of the entire track was covered with fine mesh secured to the track with cable ties attached to the outer

ring. This prevented the smallest lobsters from being able to escape under the track.

For video trials, eight of the smaller tracks were placed in one 6' diameter flow-through seawater tank at the CML. Control of the light-dark cycle was established as described for the larger tracks. In addition, a low-light camera was mounted over this tank along with an infrared light array, which made it possible to monitor lobster activity in the dark. The camera was connected to a 24-hour time lapse VCR. Tapes were changed daily. Activity was monitored for three days for each group of lobsters.

Each tape was reviewed to determine the number of times each lobster passed from one shelter to the other during each hour of the experiment. The exact timing of light changes was noted as well. For these experiments, no temperature dataloggers were used, so water temperature was obtained from records maintained by the UNH Coastal Marine Lab of daily inflow temperatures.

Data Analysis

Lobsters were separated into three size categories for the purpose of statistical comparisons (under 55, 55-65 and 65-75 mm CL). The effects of sex, size category and temperature on day and night activity were considered during analysis.

For trials involving magnetic reed-switch arrays, Boxcar files were converted to Excel format for further analysis. The difference in output voltage between shelters was used to determine when movements between shelters

occurred. This, in turn, was used to determine total laps completed by the lobster during each hour of the experimental period. For trials involving video monitoring, the number of passes from shelter to shelter each hour was counted directly.

Hourly activity data was compiled and correlated with the timing of light changes to determine how many total passes were made by lobsters during light and dark conditions. Total passes were then converted to total distance traveled (m) to allow for accurate comparison between larger and smaller racetracks. These values were averaged for one 24-hour period to determine average day distance traveled and average night distance traveled for each lobster, which were used for statistical analyses. A multivariate analysis of variance (MANOVA, Systat v.9.0, Systat Software Inc., Richmond, CA, USA) was performed to determine the effects of sex, size and ambient temperature on distance traveled.

Results

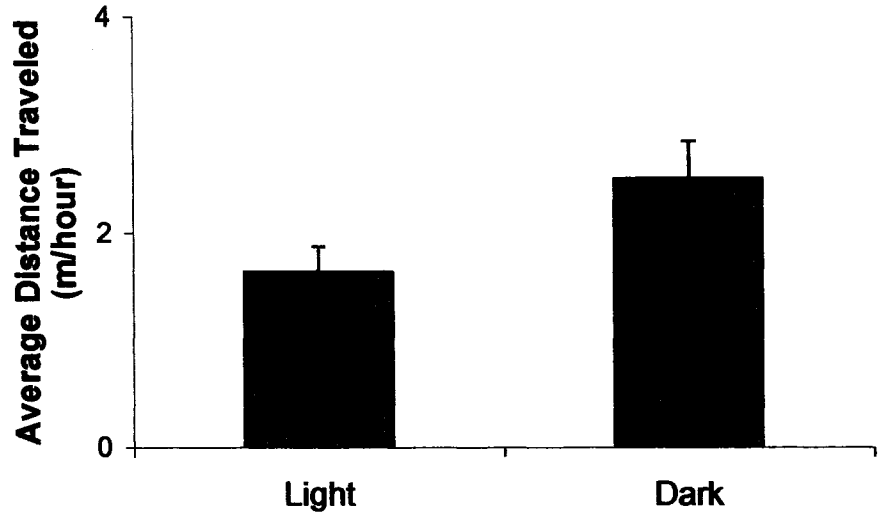
A total of 40 activity trials were completed between August, 2000 and September 2002. During the experiment, temperature ranged from 2.56 to 17.75°C on days when trials were completed. These trials included 40 lobsters (8 female and 22 male) between the sizes of 35 and 68 mm CL. Of the 40 trials, 18 were performed using the magnetic reed switch racetracks and 22 were performed using the time-lapse video racetracks. There was a significant difference between the average distances traveled per hour based on method (Systat MANOVA, $p = 0.03$). However, all winter trials were completed using the magnetic system, so there is a relationship between method and temperature. All analysis was completed first taken method into consideration as a variable and then repeated without method as a variable. Removing method as a variable never affected the significance of any other variable other than temperature.

Total distance traveled (m/day) was calculated for each lobster. One individual who moved a total of 292 m in 24 hours was identified to be an outlier and removed from further analysis.

Daily Activity Patterns

Activity as measured in passes per hour was converted to distance traveled. The average total distance traveled for all lobsters was 46.5 m/day. There was a significant difference in distance traveled based on light condition ($p< 0.001$), (Figure 4.2A). The average distance traveled per hour in the light was 1.6 ± 0.2 m and the average distance traveled in the dark was 2.5 ± 0.4 m. For

A.



B.

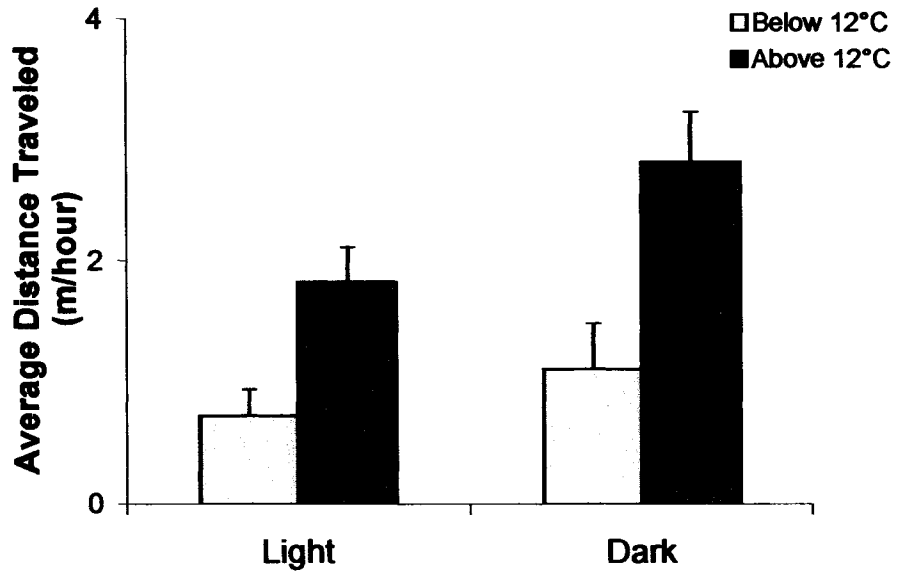


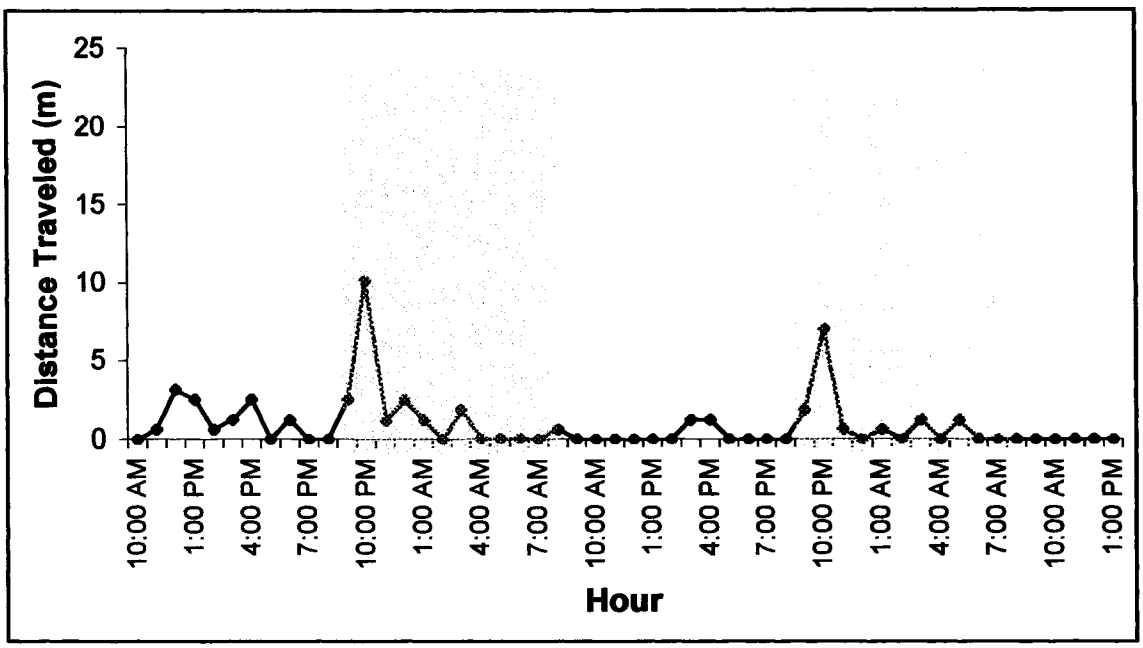
Figure 4.2. A. Comparison of average distance traveled per hour for lobsters under light and dark conditions (n = 39). Average distance traveled was significantly higher under dark conditions (2.5 ± 0.4) than under light conditions (1.6 ± 0.2) ($p = 0.03$). B. Data for average distance traveled per hour separated by temperature conditions (below 12°C, n = 7; above 12°C, n = 32). Average distance traveled was not quite significantly higher at temperatures greater than 12°C than at temperatures less than 12°C for light conditions ($p = 0.1$) and significantly higher for dark conditions ($p = 0.05$).

32 of the 39 individual lobsters used in this experiment, distance traveled was higher at night than during the day (for examples of individual activity showing nocturnal patterns, refer to Figure 4.3). One lobster showed identical distance traveled during the day and night, and 6 others traveled less distance at night than during the day.

Sources of Variability

MANOVA results revealed no significant effects of sex ($p = 0.44$) or size category ($p = 0.12$) (Figure 4.4) on distance traveled per hour for either day or night and no interaction effects ($p = 0.73$). Temperature was shown to have a significant effect on average distance traveled (m/hour), both in light ($p = 0.05$) and darkness ($p = 0.06$). Regression analysis showed that distance traveled was significantly higher at higher temperatures ($p = 0.03$). However, this relationship was not strongly linear ($r^2 = 0.12$) (Figure 4.5A). Average distance traveled (m) was not quite significantly higher at temperatures greater than 12°C than at temperatures less than 12°C for light conditions ($p = 0.1$) and significantly higher for dark conditions ($p = 0.05$) (Figure 4.2B). Total activity above 12°C is significantly higher than below 12°C ($p = 0.03$), although it is also more variable (Figure 4.5B).

A.



B.

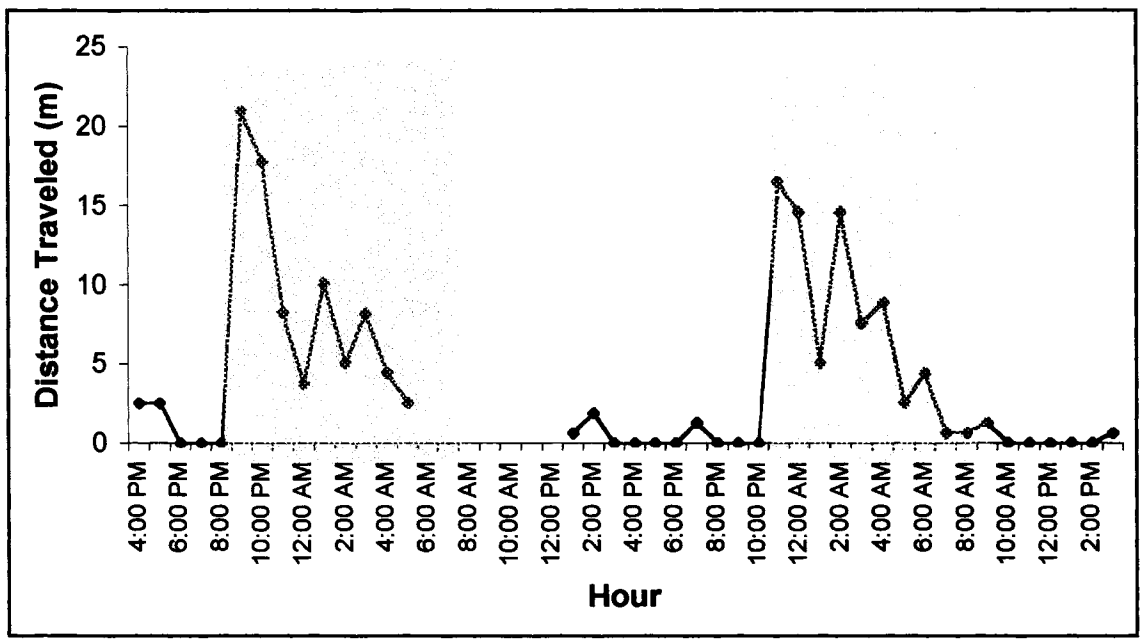


Figure 4.3. Activity in two individual lobsters. A. Recording from a lobster at ambient winter temperatures (3/14/01, 2.65°C), which exhibits overall low activity with a nocturnal rhythm and a crepuscular peak associated with the onset of darkness. B. Recording from a lobster at ambient summer temperatures (8/22/02, 16.89°C) also showing high nocturnal activity and a crepuscular peak. Both A and B show very low daytime activity levels.

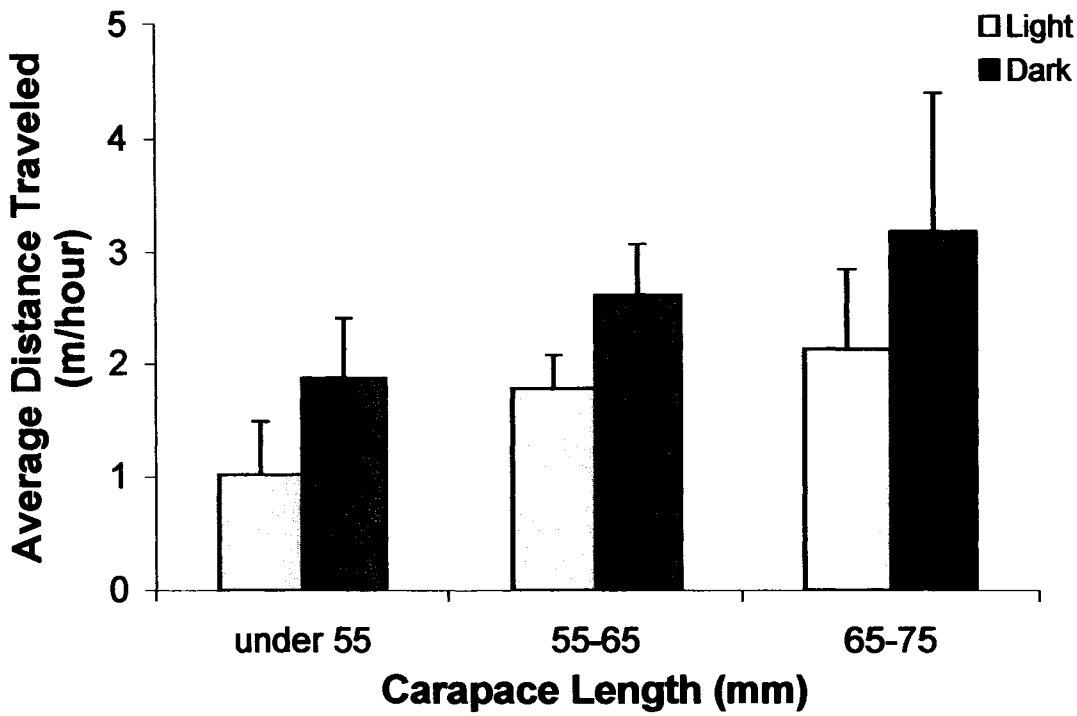


Figure 4.4. Comparison of average distance traveled per hour (m) for the three size categories of lobsters in light and dark conditions (under 55mm, n = 11; 55-65mm, n = 22, 65-75mm, n = 7). Although there is a trend for larger distances traveled by larger lobsters, there was no significant effect of size on distance traveled (p = 0.17).

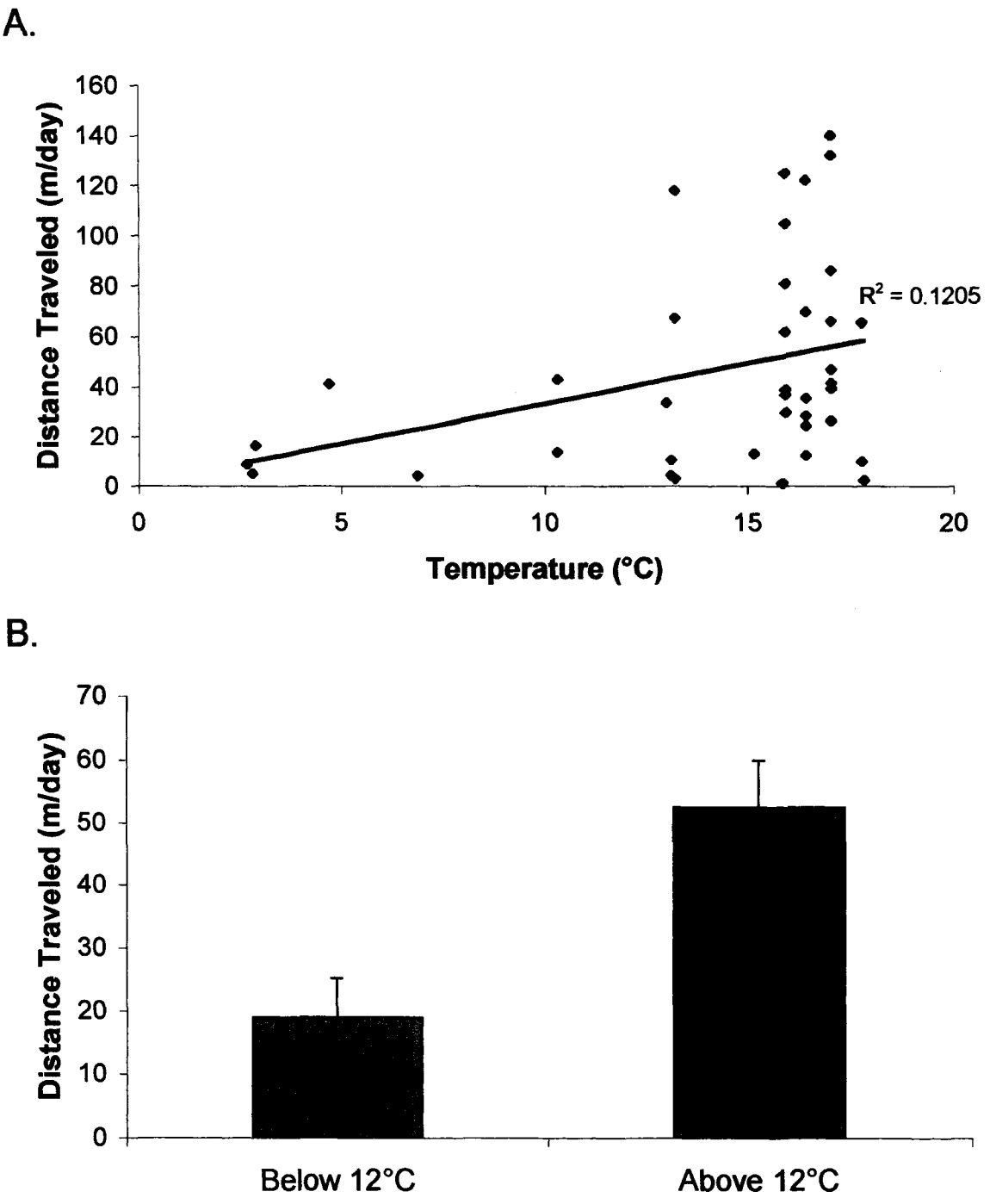


Figure 4.5. Effect of temperature on distance traveled. A. There is a significant increase in activity at higher temperatures ($p = 0.03$, $r^2 = 0.12$). B. When values for activity observed below 12°C and above 12°C are considered separately, two distinct activity states are revealed. Total activity above 12°C is significantly higher than below 12°C ($p = 0.025$), although it is also more variable.

Discussion

The lobsters used in the present study were all sub-legal size and fell within the category of "adolescent" (Steneck, 1989; Wahle and Steneck, 1991) or "juvenile" (Hudon, 1987) depending on the life history classification being referenced. Lawton and Lavalli (1995) define the transition from adolescent to adult as the onset of "functional maturity" and suggest the size of transition to be approximately 50 mm CL, which would fall within the size range of lobsters from the present study. As reproductive status was not established for lobsters in this study, I will refer to the classification schemes proposed by Steneck (1989) and Hudon (1987) and refer to these lobsters as "juveniles".

It is generally accepted that larger lobsters have larger daily activity ranges than smaller lobsters. However, there have been few studies on daily movements in sub-legal sized lobsters (<82 mm CL) lobsters. Lawton and Lavalli (1995) reported that vagile juveniles (~25-40 mm CL) might only move several meters or less daily. The one individual from the current study that fell within this size range was recorded to travel 94 m/day. Although statistical analysis was impossible due to the small sample size, this seems to indicate that at least some juveniles in this size category might travel greater distances than have been suggested.

Jury (1999) reported an average daily activity for adult lobsters (81-91 mm CL) under ambient summer temperature conditions (avg. 19.6 ± 0.27°C) to be 88 ± 12m/day (n = 18). Average daily activity for juvenile lobsters in this experiment was 50.05 m/day, which appears to supports the belief that smaller lobsters have

smaller daily activity ranges. However, direct comparisons are complicated by the larger size and temperature ranges (35-68 mm CL and 2.56 - 17.75°C) in the present study as compared to those used by Jury (1999). If only summer data from the present study is considered, the average daily activity was 52.1 m/day, which still suggests that smaller lobsters are exhibiting smaller daily activity ranges.

The primary goal of this study was to examine the effects of seasonally-appropriate ambient temperatures on juvenile lobsters. By using lobsters of both sexes and a wide range of sizes, an effort was made to elucidating ontogenic changes in the response to temperature that could partially explain observed sex ratios. In order to effectively mimic natural conditions and in consideration of the reported nocturnal status of lobster behavior, a seasonally-appropriate light/dark cycle was also imposed and the effect of light condition on activity was addressed. As has been reported for adult lobsters previously (McLeese and Wilder, 1958; Herrnkind, 1980; Aiken and Waddy, 1986; Factor 1995, Jury 1999) temperature was shown to have a significant affect on activity. The results of this study indicate few, if any, ontogenic changes in the response to temperature in the lobster. There was no effect of size or sex on activity levels over the range of sizes examined in this study. In fact, juvenile lobsters exhibited remarkably similar activity responses to temperature as compared to results from adult lobsters reported previously (Jury, 1999). Although there was a trend for higher activity at higher temperatures, the correlation was not linear (Figure 4.4). Rather, the data supports the existence of the "activity states" proposed by Jury

(1999), with consistently low activity below 12°C and higher but also more variable activity at temperatures greater than 12°C.

Although temperature is clearly a pervasive influence on lobster behavior and physiology, light levels have been repeated shown to be the most influential abiotic factor in regards to activity levels (Cobb, 1969; Zeitllan-Hale and Sastry, 1978; Lawton, 1987; Lawton and Lavalli, 1995, Jury, 1999). Other than temperature, the only variable with a significant effect on activity was light condition. Activity levels for juvenile lobsters, as measured in distance traveled per hour, were significantly higher in dark conditions than in light (Figure 4.2). This nocturnal activity pattern is similar to what has been reported previously for adult lobsters (Cobb, 1969; Cooper and Uzmann, 1980; Lawton and Lavalli, 1995; Jury, 1999). Close examination of the activity records of several individual lobsters (Figure 4.3) reveals a crepuscular component to the nocturnal activity pattern. Although overall activity was affected by temperature and therefore season (Figure 4.3a and 4.3b), most lobsters showed a peak of activity associated with the onset of the dark condition regardless of temperature. This also agrees with what was found previously for adult lobsters (Jury, 1999).

In conclusion, temperature and light condition were found to exert a controlling influence over activity levels as measured by distance traveled in juvenile lobsters. No significant effect of sex or size was observed for the range of sizes examined in this study. However, the results of this study, when compared to previous work on larger lobsters, suggest that smaller lobsters may have smaller daily activity ranges than larger lobsters.

CHAPTER V

CONCLUSIONS AND FUTURE DIRECTIONS

Heart rate in lobsters exhibits a linear increase with increasing temperature from 5 to 25°C. The effect of temperature on isolated hearts was examined in order to determine how temperature influenced the heart in the absence of exogenous inputs from neural or endocrine sources. Isolated hearts taken from lobsters acclimated to 15°C showed no significant increase in beat rate in response to increased temperature from 5 to 20°C. Thus, it is likely that an exogenous input was responsible for the increase in rate observed in intact animals.

This increase is not caused by the cardioregulatory (CR) nerves, as lesioning these nerves resulted in no significant change in the effect of temperature on heart rate. Circulating neurohormones released by the pericardial organs are the likely source of the temperature-induced increase in heart rate. To determine which hormone or hormones are responsible, it would be necessary to determine which secretions of the pericardial organ increase with increasing temperature and then to independently assess the effects of these hormones on heart function, either *in vitro* or *in vivo*.

Most isolated hearts failed between 20 and 25°C. This failure may have been initiated by the decline in ambient dissolved oxygen as temperature

increased, or by the loss of hormonal inputs that facilitate oxygen or nutrient uptake by the cells of the cardiac ganglion. Once temperature-induced changes in hormone concentrations have been assessed, it would be possible to examine the effects of these hormones on the longevity of isolated hearts exposed to higher temperatures. Also, in future work, acclimation condition of the lobster from which the heart is taken should be considered.

Lobsters exhibited an avoidance response when water temperature was increased by 9.4°C +/- 1.1 in ambient winter conditions (~5°C) and 7.5 +/- 0.6°C in ambient summer conditions (~15°C). The temperature at which avoidance occurred was significantly higher in summer than in winter (22.3 +/-0.5°C and 14.3 +/- 1.1°C, respectively; $p = 0.001$), but there was no significant difference in the change in temperature required to elicit avoidance by season. Lobsters acclimated to winter temperatures exhibited avoidance at a temperature previously shown to be within the preferred thermal range for the species (14.26°C). This suggests that, regardless of acclimation condition, an increase of 7-9°C in ambient temperature is aversive, possibly because the associated increase in metabolic rate approaches the limits of the aerobic scope of the lobster. This is supported by previous work that suggests avoidance behaviors can be related to the metabolic costs associated with adaptation to a new thermal regime (Claireaux *et al.*, 1995). This is further supported by the results presented previously from the isolated heart experiments, in which hearts failed after a similar increase in ambient temperature. Future avoidance studies in lobsters should consider metabolism more directly by monitoring dissolved

oxygen concentration and oxygen consumption as temperatures increase or by artificially lowering dissolved oxygen concentration to determine if avoidance temperature is decreased.

Juvenile lobsters exhibited a clear nocturnal activity pattern, possibly with a crepuscular peak at dusk, with significantly higher activity in dark conditions than in light conditions ($p < 0.001$). Temperature had a significant effect on activity levels in both light and dark conditions ($p = 0.05$ and 0.06, respectively), with higher activity at higher temperatures. However, this relationship was not linear. Similar to what was observed by Jury (1999) with adult lobsters, there appeared to be two distinct "activity states". Total activity above 12°C is significantly higher than below 12°C ($p = 0.03$), although it is also more variable. To eliminate the possible influences of other seasonal variables, such as day length, on activity states, it would be beneficial in the future to examine activity in winter-acclimated individuals held at artificially maintained summer temperatures and vice versa.

One of the main goals of these studies was to elucidate the role of temperature in seasonal migrations of the lobsters in the Great Bay estuary system. The lobsters are known to move into the estuary in late spring or early summer, after the low salinity period associated with snow melt run off and spring storms has passed. At this time, the temperature of the estuary is at least slightly greater than that observed at the coast (Figure 1.2) and would be approaching the low end of the range for the higher activity state (Figure 4.4). This could result in a tendency for lobsters to move away from the cooler coastal waters and into

the estuary. Migration out of the estuary begins in mid- to late summer, when estuarine temperatures are still high. However, peak temperatures in the upper estuary can exceed 22°C at this time, which is approximately the temperature of avoidance observed for summer acclimated animals (Figure 3.5). Avoidance of high temperatures in the upper estuary, combined with a relatively uniform temperature between the coast and the estuary in the fall, could result in a tendency for lobsters to move out of the estuary and return to coastal locations.

The results of these studies only provide a partial explanation for the skewed sex-ratio observed in the estuary, as few sex-based differences were observed. Males were observed to have a significantly higher avoidance temperature than females during summer, which could lead them to move farther into the estuary where temperatures are higher, but these data do not fully explain the ratios observed. It is possible that females with attached eggs are remaining in coastal waters to release the eggs and therefore entering the estuary later in the summer. However, avoidance thresholds and activity levels of ovigerous females were not established in these studies. Future work should consider reproductive condition of females.

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